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APPLICATION NUMBER: 60/532,130

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Additional inventors are being named on the 1 separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

ANTI-IL13 ANTIBODIES AND USES THEREOF

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages **58**

☐ CD(s), Number

☒ Drawing(s) Number of sheets **10**

☐ Other

Sequence Listing

☐ Application Data Sheet. See 37 CFR 1.76

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Respectfully submitted,

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Docket Number

45,275

TNX1050

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Anti-IL13 Antibodies and Uses Thereof

Background of Invention

[0001] The interleukin (IL)-13 is a pleiotropic T helper cell subclass 2 (Th2) cytokine. Like IL4, IL13 belongs to the family of type I cytokines sharing the tertiary structure defined by a 4 α -helical hydrophobic bundle core. IL13 has approximately 30% amino acid sequence homology with IL4 and shares many of the properties of IL4 (Wynn, *Ann. Rev. Immunol.*, 21: 425 (2003)). The functional similarity of IL4 and IL13 is attributed to the fact that IL13 can bind IL4 receptor alpha chain (IL4R- α) subsequent to its binding to IL13 receptor alpha chain-1 (IL13R α 1) (Hershey, *J. Allergy Clin. Immunol.*, 111: 677 (2003)). IL4R α is activated by IL4 and IL13 resulting in Jak1-dependent STAT6 phosphorylation. Both IL4 and IL13 promote B-cell proliferation and induce class switching to IgG4 and IgE in combination with CD40/CD40L costimulation (Punnonen et al., *Proc. Natl. Acad. Sci. USA*, 90: 3730 (1993), Oettgen et al., *J. Allergy Clin. Immunol.*, 107: 429 (2001)).

[0002] However, unlike IL4, IL13 is not involved in the differentiation of naïve T cells into Th2 cells (Zurawski et al., *Immunol. Today*, 15: 19 (1994)). IL13 up-regulates Fc ϵ RI and thus helps in IgE priming of mast cells (de Vries, *Allergy Clin. Immunol.* 102: 165 (1998). In monocytes/macrophages, IL13 up-regulates expression of CD23 and MHC class I and class II antigens, down-regulate the expression of Fc γ and CD14, and inhibit antibody-dependent cytotoxicity (de Waal Malefyt et al., *J. Immunol.*, 151: 6370 (1993), Chomarat et al., *Int. Rev. Immunol.*, 17: 1 (1998)). IL13, but not IL4, promotes eosinophil survival, activation, and recruitment (Horie et al., *Intern. Med.*, 36: 179 (1997), Luttmann et al., *J. Immunol.* 157: 1678 (1996), Pope et al., *J. Allergy Clin. Immunol.*, 108: 594 (2001). IL13 also manifests important functions on nonhematopoietic cells, such as smooth muscle cells, epithelial cells, endothelial cells and fibroblast cells. IL13 enhances proliferation and cholinergic-induced contractions of smooth muscles (Wills-Karp, *J. Allergy Clin. Immunol.*, 107: 9 (2001). In epithelial cells IL13 is a potent inducer of chemokine production (Li et al., *J. Immunol.*, 162: 2477 (1999), alters mucociliary differentiation (Laoukili et al., *J. Clin. Invest.*, 108: 1817 (2001), decreases ciliary beat frequency of ciliated epithelial cells (Laoukili et al., *J. Clin. Invest.*, 108: 1817 (2001), and results in goblet cell metaplasia (Zhu et al., *J. Clin. Invest.*, 103: 779 (1999), Grunig et al., *Science*, 282: 2261 (1998)). In endothelial cells IL13 is a potent inducer of vascular cell adhesion molecule 1 (VCAM-1) which is important for recruitment of eosinophils (Bochner et

al., *J. Immunol.*, 154: 799 (1995)). In human dermal fibroblasts IL13 induces type 1 collagen synthesis in human dermal fibroblasts (Roux et al., *J. Invest. Dermatol.*, 103: 444 (1994)).

[0003] Although IL13 and IL4 share certain functional similarities, studies in animal models of disease and gene-knockout mice demonstrated that IL13 possesses unique effector functions distinct from IL4 and provides compelling evidence that IL13, independent of other Th2 cytokines, is necessary and sufficient to induce all features of allergic asthma (Wills-Karp et al. *Science*, 282: 2258 (1998), Walter et al. *J. Immunol.* 167: 4668 (2001)). IL13 may play a more significant role than other Th2 cytokines in effector functions associated with the symptoms of asthma (Corry, *Curr. Opin. Immunol.*, 11: 610 (1999)). This contention is supported in human disease by a strong association between IL13 levels and genetic polymorphisms in the IL13 gene and disease correlates (Wills-Karp. et al. *Respir. Res.* 1: 19 (2000); Vercelli et al., *Curr. Opin. Allergy Clin. Immunol.*, 2: 389 (2002); He et al., *Genes Immunol.*, 4: 385 (2003), Arima et al., *J. Allergy Clin. Immunol.*, 109: 980 (2003), Liu et al., *J. Clin. Allergy Immunol.*, 112: 382 (2003)). Emerging data suggest that IL13 induces features of the allergic response via its actions on mucosal epithelium and smooth muscle cells, rather than through the traditional pathways involving eosinophils and IgE-mediated events (Wills-Karp et al., *Sci.*, 282: 2258 (1998)).

[0004] Asthma is described as a chronic pulmonary disease that involves airway inflammation, hyperresponsiveness and obstruction. Physiologically, airway hyperresponsiveness is documented by decreased bronchial airflow after bronchoprovocation with methacholine or histamine. Other triggers that provoke airway obstruction include cold air, exercise, viral upper respiratory infection, cigarette smoke, and respiratory allergens. Bronchial provocation with allergen induces a prompt early phase immunoglobulin E (IgE)-mediated decrease in bronchial airflow followed in many patients by a late-phase IgE-mediated reaction with a decrease in bronchial airflow for 4-8 hours. The early response is caused by acute release of inflammatory substances, such as histamine, PGD₂, leukotriene, tryptase and platelet activating factor (PAF), whereas the late response is caused by de novo synthesized pro-inflammatory cytokines (e.g. TNF α , IL4, IL13) and chemokines (e.g. MCP-1 and MIP-1 α) (Busse et al. In: *Allergy: Principles and Practice*, Ed. Middleton, 1173 (1998)). In chronic asthmatic patients, persistent pulmonary symptoms are mediated by the heightened response of Th2 cells. Th2 cytokines are believed to play a vital role in the disease (Larche et al., *J. Allergy Clin. Immunol.*, 111: 450 (2003)), in particular, IL13 and IL4 produced by Th2 cells with NK phenotype (NKT) in the airway as indicated in a model of asthma in rodents (Akbari et al., *Nature Med.*, 9: 582 (2003)). The gross pathology of asthmatic airways displays lung hyperinflation, smooth muscle hypertrophy, lamina reticularis thickening, mucosal edema,

epithelial cell sloughing, cilia cell disruption, and mucus gland hypersecretion. Microscopically, asthma is characterized by the presence of increased numbers of eosinophils, neutrophils, lymphocytes, and plasma cells in the bronchial tissues, bronchial secretions, and mucus. Initially, there is recruitment of leukocytes from the bloodstream to the airway by activated CD4+ T-lymphocytes. The activated T-lymphocytes also direct the release of inflammatory mediators from eosinophils, mast cells, and lymphocytes. In addition, the Th2 cells produces IL4, IL5, IL9 and IL13. IL4, in conjunction with IL13, signals the switch from IgM to IgE antibodies.

[0005] Cross-linking of membrane-bound IgE molecules by allergen causes mast cells to degranulate, releasing histamine, leukotrienes, and other mediators that perpetuate the airway inflammation. IL5 activates the recruitment and activation of eosinophils. The activated mast cells and eosinophils also generate their cytokines that help to perpetuate the inflammation. These repeated cycles of inflammation in the lungs with injury to the pulmonary tissues followed by repair may produce long-term structural changes ("remodeling") of the airways.

[0006] Moderate asthma is currently treated with a daily inhaled anti-inflammatory-corticosteroid or mast cell inhibitor such as cromolyn sodium or nedocromil plus an inhaled beta2-agonist as needed (3-4 times per day) to relieve breakthrough symptoms or allergen- or exercise-induced asthma. Cromolyn sodium and nedocromil block bronchospasm and inflammation, but are usually effective only for asthma that is associated with allergens or exercise and typically, only for juvenile asthmatics. Inhaled corticosteroids improve inflammation, airways hyperreactivity, and obstruction, and reduce the number of acute exacerbations. However, it takes at least a month before effects are apparent and up to a year for marked improvement to occur. The most frequent side effects are hoarseness and oral fungal infection, i.e., candidiasis. More serious side effects have been reported, e.g., partial adrenal suppression, growth inhibition, and reduced bone formation, but only with the use of higher doses. Beclomethasone, triamcinolone, and flunisolide probably have a similar potency; whereas budesonide and fluticasone are more potent and reportedly have fewer systemic side effects.

[0007] Even patients with mild disease show airway inflammation, including infiltration of the mucosa and epithelium with activated T cells, mast cells, and eosinophils. T cells and mast cells release cytokines that promote eosinophil growth and maturation and the production of IgE antibodies, and these, in turn, increase microvascular permeability, disrupt the epithelium, and stimulate neural reflexes and mucus-secreting glands. The result is airways hyperreactivity, bronchoconstriction, and hypersecretion, manifested by wheezing, coughing, and dyspnea.

- [0008] Traditionally, asthma has been treated with oral and inhaled bronchodilators. These agents help the symptoms of asthma, but do nothing for the underlying inflammation. Recognition during the last 10 years of the importance of inflammation in the etiology of asthma has led to the increased use of corticosteroids, but many patients continue to suffer from uncontrolled asthma.
- [0009] Because of the importance of treating inflammatory diseases in humans, particularly asthma, new bioactive compounds having fewer side effects are continually being sought. The development of potent and specific inhibitors of IL13, which remain active when administered long term to asthmatic airways, offers a novel approach to the treatment of asthma, as well as in other IL13- and IgE-mediated diseases.

Summary of Invention

- [0010] The present invention relates to antibodies that bind specifically and with high affinity to both glycosylated and non-glycosylated human IL13; does not bind mouse IL13, and neutralize human IL13 activity at an approximate molar ratio of 1:2 (mAb:IL13). Also included in the present invention are antibodies comprising the antigen binding regions derived from the light and heavy chain variable regions of said antibody. The antibodies of the invention may be monoclonal, and the monoclonal antibody may be a human antibody, a chimeric antibody, or a humanized antibody.
- [0011] Examples of these antibodies are 228B/C-1, 228A-4, 227-26, and 227-43. The hybridomas that produce these antibodies were deposited on November 20, 2003, with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, under Accession Numbers PTA-5657, PTA-5656, PTA-5654, and PTA-5655, respectively (See Biological Deposit Data Below).
- [0012] The present invention includes antibodies which have a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 3, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 4; antibodies which have a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 5, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 6; and antibodies which have a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 7, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 8. The present invention also includes a recombinant antibody molecule, or an IL13-binding fragment thereof, comprising at least one antibody heavy chain,

or an IL13-binding fragment thereof, comprising non-human CDRs at positions 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3) (Kabat numbering) from a mouse anti-IL13 antibody, wherein positions 27-30 have the amino acid Gly 26, Phe 27, Ser 28, Leu 29, Asn 30, (SEQ ID NO: 18); and at least one antibody light chain, or an IL13-binding fragment thereof, comprising non-human CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3) from a mouse anti-IL13 antibody, and framework regions from a human monoclonal antibody.

- [0013] The present invention includes the hybridoma cell lines that produce the monoclonal antibodies 228B/C-1, 228A-4, 227-26, and 227-43. The present invention includes the nucleic acids encoding monoclonal antibodies 228B/C-1, 228A-4, 227-26, and 227-43, cell lines comprising a nucleic acid encoding these antibodies, and vectors comprising the nucleic acid encoding these antibodies.
- [0014] The present invention includes a composition comprising the antibodies according to the claimed invention in combination with a pharmaceutically acceptable carrier, diluent, excipient, or stabilizer.
- [0015] The present invention includes a method of treating a subject suffering from asthmatic symptoms comprising administering an amount of an antibody according to the claimed invention effective to reduce the asthmatic symptoms, wherein the antibody may down-regulate the activity of IL13 in the patient, reduce bronchial hyperresponsiveness in the patient, and/or reduce eosinophilia in the lungs of the subject. The present invention also includes a method of inhibiting the infection of respiratory syncytial virus (RSV) comprising administering an inhibiting amount of the antibody of the claimed invention.
- [0016] The antibody of the present invention may be administered by one or more of the routes including intravenous, intraperitoneal, inhalation, intramuscular, subcutaneous and oral routes. The present invention includes an inhalation device that delivers to a patient a therapeutically effective amount of an antibody according to the claimed invention.
- [0017] The present invention includes a method for detecting interleukin-13 protein in a patient suffering from an allergic disease, comprising the steps of allowing the antibody of the claimed invention to contact a sample; and detecting the interleukin-13 through the occurrence of immunoreaction and a method for diagnosing overexpression of IL13 in a subject, comprising the steps of (a) obtaining a sample from the subject; (b) combining the sample with an antibody according to the claimed invention under conditions which would allow

immunoreaction with IL13; and (c) determining whether or not IL13 is overexpressed relative to a normal level of expression of IL13.

- [0018] The present invention includes a method for producing the antibodies of the claimed invention, comprising the steps of: a) producing an immunogenic compound comprising a glycosylated IL13 moiety and an immunogenic moiety; b) preparing an injectable solution comprising said immunogenic compound in phosphate buffered saline (PBS) and an adjuvant; c) immunizing a mouse with said injectable solution by a combination of intravenous and intraperitoneal injections, d) producing a hybridoma by fusing a spleen cell from said immunized mouse with a myeloma cell; e) selecting a hybridoma producing an antibody having the characteristics of the antibody of the claimed invention; and f) isolating said antibody.
- [0019] The present invention includes a method for inhibiting IgE antibody production in a patient, which comprises administering to the patient an IgE antibody production inhibiting effective amount of an antibody according to the claimed invention. The inhibition of IgE antibody production may prevent bronchial asthma, allergic rhinitis, allergic dermatitis, and anaphylaxis, and also treat bronchial asthma, allergic rhinitis, urticaria, and atopic dermatitis.
- [0020] The present invention includes a method of treating an IL13-mediated disorder in a patient, comprising administering to the patient an effective amount of an antibody or antigen-binding fragment thereof according to the claimed invention, wherein said antibody or antigen-binding fragment thereof inhibits binding of IL13 to its receptor and inhibits one or more functions associated with binding of the interleukin to said receptor.
- [0021] The present invention includes a method of treating an IgE-mediated disorder in a patient, comprising administering to the patient an effective amount of an antibody or antigen-binding fragment thereof according to the claimed invention, wherein said antibody or antigen-binding fragment thereof inhibits binding of IL13 to its receptor and inhibits one or more functions associated with binding of the interleukin to said receptor.
- [0022] The present invention includes a method for reducing the severity of asthma in a mammal comprising administering to the mammal a therapeutically effective amount of an anti-IL13 monoclonal antibody having the following characteristics; ability to bind human IL13 with a K_D between about 1×10^{10} to about 1×10^{12} M, ability to inhibit one or more functions associated with binding of the interleukin IL13 to the IL13 receptor; and the antibody does not bind mouse IL13.

[0023] Diseases and/or conditions mediated by IL13 that are contemplated by the invention include, but are not limited to, allergic asthma, non-allergic (intrinsic) asthma, allergic rhinitis, atopic dermatitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, and osteoporosis.

Brief Description of Drawings

[0024] FIGURE 1 depicts the binding of anti-IL13 monoclonal antibodies to human IL13.

[0025] FIGURE 2 depicts the binding of anti-IL13 monoclonal antibodies mutant IL13-Fc.

[0026] FIGURE 3 illustrates that there is no inhibition of mAb 228B/C-1 binding to human IL13 by mAb JES10-5A2 (Pharmingen).

[0027] FIGURE 4 illustrates the effect of anti-IL13 monoclonal antibodies on the proliferation of Hodgkin Lymphoma L-1236 cells.

[0028] FIGURE 5 illustrates the effect of anti-IL13 monoclonal antibodies on IL13-induced suppression of CD14 expression in human monocytes.

[0029] FIGURE 6 illustrates the effect of anti-IL13 monoclonal antibodies on IL13-induced up-regulation of CD23 expression in human monocytes.

[0030] FIGURE 7 illustrates the effect of anti-IL13 monoclonal antibodies on IL13-induced STAT6 phosphorylation in THP-1 cells.

[0031] FIGURE 8 depicts the amino acid sequence of the VH and VL regions of monoclonal antibody 228B/C-1.

[0032] FIGURE 9 depicts the amino acid sequence of the VH and VL regions of monoclonal antibody 228A-4.

[0033] FIGURE 10 depicts the amino acid sequence of the VH and VL regions of monoclonal antibody 227-26.

Detailed Description

Biological Deposit Data

Deposit Date: 20 November 2003

Deposit Term: 30 Years

Tanox, Inc.

10301 Stella Link

Houston, TX

77025

United States of America

Deposit Accession Number: PTA-5657

Deposit Description: mouse monoclonal hybridoma cell line 228B/C-1

Deposit Accession Number: PTA-5656

Deposit Description: mouse monoclonal hybridoma cell line 228A-4

Deposit Accession Number: PTA-5654

Deposit Description: mouse monoclonal hybridoma cell line 227-26

Deposit Accession Number: PTA-5655

Deposit Description: mouse monoclonal hybridoma cell line 227-43

[0034] This invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described herein because they may vary. Further, the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention. As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise, e.g., reference to "a host cell" includes a plurality of such host cells.

[0035] Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of the invention. Although any methods and materials similar or equivalent to those

described herein can be used in the practice of the present invention, the methods, devices, and materials are described herein.

[0036] All patents and publications mentioned herein are incorporated herein by reference to the extent allowed by law for the purpose of describing and disclosing the proteins, enzymes, vectors, host cells, and methodologies reported therein that might be used with the present invention. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

IMMUNOGEN

[0037] Recombinant IL13 is commercially available from a number of sources (see, e.g. R & D Systems, Minneapolis, MN., PeproTech, Inc., NJ, and Sanofi Bio-Industries, Inc., Tervose, PA.). Alternatively, a gene or a cDNA encoding IL13 may be cloned into a plasmid or other expression vector and expressed in any of a number of expression systems according to methods well known to those of skill in the art. Methods of cloning and expressing IL13 and the nucleic acid sequence for IL13 are well known (see, for example, U.S. Patent No. 5,652,123). Because of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding IL13 polypeptides may be produced. One may vary the nucleotide sequence by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence that codes for naturally occurring IL13 polypeptide and all such variations are to be considered. Any one of these polypeptides may be used in the immunization of an animal to generate antibodies of the present invention.

[0038] A fusion protein comprising a mutant form of human IL13 was used to generate the antibodies of the present invention. This mutant form of IL13 contained a single mutation resulting in an inactive form of the protein (Thompson et al., J. Biol. Chem. 274: 2994 (1999)). In order to generate neutralizing antibodies with high affinity, the fusion protein comprised the mutant IL13 protein fused to an immunoglobulin Fc, specifically IgG1, and was expressed in a mammalian cell line such that the recombinant protein was naturally glycosylated. The Fc portion of the fusion protein may have provided a conformational structure that exposed a key epitope. The glycosylation may have increased the immunogenicity of the epitope, allowing the generation of antibodies to this particular epitope.

[0039] IL13 polypeptides expressed in *E. coli* lack glycosylation and the commercially available antibodies tested were generated using this protein. We tested these antibodies, e.g., R&D

Systems and Pharmingen, and found that they do not cross react with the epitope bound by the antibodies of the present invention.

ANTAGONIST ANTIBODIES

[0040] The present invention provides antagonist antibodies that inhibit and neutralize the action of IL13. In particular, the antibodies of the present invention bind to IL13 inhibiting the binding of IL13 to the IL13 receptor alpha chain -1 (IL13R α 1) blocking the activation of the receptor. The antibodies of the present invention may be polyclonal or monoclonal, and may be chimeric, human, humanized, or a binding fragment thereof.

ANTIBODY GENERATION

[0041] The antibodies of the present invention may be generated by any suitable method known in the art. The antibodies of the present invention may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan (Harlow, et al., *Antibodies: a Laboratory Manual*, (Cold spring Harbor Laboratory Press, 2nd ed. (1988), which is hereby incorporated herein by reference in its entirety).

[0042] For example, an immunogen as described above may be administered to various host animals including, but not limited to, rabbits, mice, rats, etc., to induce the production of sera containing polyclonal antibodies specific for the antigen. The administration of the immunogen may entail one or more injections of an immunizing agent and, if desired, an adjuvant. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Additional examples of adjuvants which may be employed includes the MPL-TDM adjuvant (monophosphoryl lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation. Such adjuvants are also well known in the art.

[0043] Typically, the immunogen and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections, though they may also be given intramuscularly, and/or through IV. The immunogen may include an IL13 polypeptide or a fusion protein or variants thereof. Depending upon the nature of the polypeptides (i.e., percent hydrophobicity,

percent hydrophilicity, stability, net charge, isoelectric point etc.), it may be useful to conjugate the immunogen to a protein known to be immunogenic in the mammal being immunized. Such conjugation includes either chemical conjugation by derivatizing active chemical functional groups to both the polypeptide of the present invention and the immunogenic protein such that a covalent bond is formed, or through fusion-protein based methodology, or other methods known to the skilled artisan. Examples of such immunogenic proteins include, but are not limited to keyhole limpet hemocyanin, ovalbumin, serum albumin, bovine thyroglobulin, soybean trypsin inhibitor, and promiscuous T helper peptides. Various adjuvants may be used to increase the immunological response as described above.

[0044] The antibodies of the present invention may comprise monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975) and U.S. Pat. No. 4,376,110, by Harlow, et al., *Antibodies: A Laboratory Manual*, (Cold spring Harbor Laboratory Press, 2nd ed. (1988), by Hammerling, et al., *Monoclonal Antibodies and T-Cell Hybridomas* (Elsevier, N.Y., (1981)), or other methods known to the artisan. Other examples of methods which may be employed for producing monoclonal antibodies include, but are not limited to, the human B-cell hybridoma technique (Kosbor et al., 1983, *Immunology Today* 4:72; Cole et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies And Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated in vitro or in vivo.

[0045] In a hybridoma method, a mouse, a humanized mouse, a mouse with a human immune system, hamster, or other appropriate host animal, is typically immunized with an immunogen to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to IL13. Alternatively, lymphocytes may be immunized in vitro with the antigen.

[0046] Generally, in making antibody-producing hybridomas, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986), pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that

preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

- [0047] Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, Calif. and the American Type Culture Collection, Manassas, Va. As inferred throughout the specification, human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63).
- [0048] The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the IL13. The binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoadsorbant assay (ELISA). Such techniques are known in the art and within the skill of the artisan. The binding affinity of the monoclonal antibody to IL13 can, for example, be determined by the Scatchard analysis (Munson et al., Anal. Biochem., 107:220 (1980)).
- [0049] After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, supra). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640.
- [0050] The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-sepharose, hydroxyapatite chromatography, gel exclusion chromatography, gel electrophoresis, dialysis, or affinity chromatography.
- [0051] A variety of methods exist in the art for the production of monoclonal antibodies and thus, the invention is not limited to their sole production in hybridomas. For example, the monoclonal antibodies may be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567. In this context, the term "monoclonal antibody" refers to an antibody derived

from a single eukaryotic, phage, or prokaryotic clone. The DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies, or such chains from human, humanized, or other sources). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transformed into host cells such as NS0 cells, Simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; Morrison et al, supra) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

[0052] The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain cross-linking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent cross-linking.

[0053] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

[0054] For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Pat. Nos. 5,807,715; 4,816,567;

and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework (FR) regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entirety). Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332). Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the methods of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Reichmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted from analogous sites in rodent antibodies.

[0055] In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988)¹ and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992).

[0056] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety. The techniques of Cole et al., and Boerder et al., are also available for the preparation of human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Riss, (1985); and Boerner et al., *J. Immunol.*, 147(1):86-95, (1991)).

[0057] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, Calif.), Genpharm (San Jose, Calif.), and Medarex, Inc.

(Princeton, N.J.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

- [0058] Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and creation of an antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,106, and in the following scientific publications: Marks et al., *Biotechnol.*, 10:779-783 (1992); Lonberg et al., *Nature* 368:856-859 (1994); Fishwild et al., *Nature Biotechnol.*, 14:845-51 (1996); Neuberger, *Nature Biotechnol.*, 14:826 (1996); Lonberg and Huszer, *Intern. Rev. Immunol.*, 13:65-93 (1995).
- [0059] Also human mAbs could be made by immunizing mice transplanted with human peripheral blood leukocytes, splenocytes or bone marrows (e.g. Trioma techniques of XTL). Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).
- [0060] Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.
- [0061] The antibodies of the present invention may be bispecific antibodies. Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present invention, one of the binding specificities may be directed towards IL13, the other may be for any other antigen, and preferably for a cell-surface

protein, receptor, receptor subunit, tissue-specific antigen, virally derived protein, virally encoded envelope protein, bacterially derived protein, or bacterial surface protein, etc.

- [0062] Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published May 13, 1993, and in Traunecker et al., *EMBO J.*, 10:3655-3659 (1991).
- [0063] Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It may have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transformed into a suitable host organism. For further details of generating bispecific antibodies see, for example Suresh et al., *Meth. In Enzym.*, 121:210 (1986).
- [0064] Heteroconjugate antibodies are also contemplated by the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980). It is contemplated that the antibodies may be prepared in vitro using known methods in synthetic protein chemistry, including those involving cross-linking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioester bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Pat. No. 4,676,980.

IDENTIFICATION OF ANTI-IL13 ANTIBODIES

- [0065] Candidate anti-IL13 antibodies were tested by enzyme linked immunosorbent assay (ELISA), Western immunoblotting, or other immunochemical techniques. Assays performed to characterize the individual antibodies included: (1) Inhibition of IL13-autocrine proliferation of

Hodgkin's lymphoma cell lines HDLM-2 and L-1236; (2) Inhibition of IL13-induced STAT6 phosphorylation in THP-1 cells; and (3) Inhibition of IL13-induced suppression of CD14 expression in primary human monocytes; and (4) Inhibition of IL13-induced up-regulation of CD23 expression on primary human monocytes. Experimental details are described in the Examples.

[0066] Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, monovalent, bispecific, heteroconjugate, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above.

[0067] The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. Moreover, the term "antibody" (Ab) or "monoclonal antibody" (mAb) is meant to include intact molecules, as well as, antibody fragments (such as, for example, Fab and F(ab')₂ fragments) which are capable of specifically binding to protein. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation of the animal or plant, and may have less non-specific tissue binding than an intact antibody (Wahl et al., J. Nucl. Med. 24:316-325 (1983)).

[0068] The antibodies may be human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, non-human primates, rodents (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin

and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati et al.

- [0069] The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of IL13 or may be specific for both IL13 as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).
- [0070] Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of IL13 which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures.
- [0071] Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that bind IL13 polypeptides, which have at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to IL-13 are also included in the present invention.
- [0072] In specific embodiments, antibodies of the present invention cross-react with monkey homologues of human IL13 and the corresponding epitopes thereof. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of the specific antigenic and/or immunogenic polypeptides disclosed herein.
- [0073] Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide encoding IL13 under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with an equilibrium dissociation constant or K_D less than 5×10^{-9} M; 10^{-9} M; 5×10^{-10} M; 10^{-10} M; 5×10^{-11} M; 10^{-11} M; 5×10^{-12} M; 10^{-12} M; 5×10^{-13} M; 10^{-13} M; 5×10^{-14} M; 10^{-14} M; 5×10^{-15} M; 10^{-15} M. The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example,

the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

VECTORS AND HOST CELLS

- [0074] In another aspect, the present invention provides vector constructs comprising a nucleotide sequence encoding the antibodies of the present invention and a host cell comprising such a vector. Standard techniques for cloning and transformation may be used in the preparation of cell lines expressing the antibodies of the present invention.
- [0075] Recombinant expression vectors containing a nucleotide sequence encoding the antibodies of the present invention can be prepared using well known techniques. The expression vectors include a nucleotide sequence operably linked to suitable transcriptional or translational regulatory nucleotide sequences such as those derived from mammalian, microbial, viral, or insect genes. Examples of regulatory sequences include transcriptional promoters, operators, enhancers, mRNA ribosomal binding sites, and appropriate sequences which control transcription and translation initiation and termination. Nucleotide sequences are "operably linked" when the regulatory sequence functionally relates to the nucleotide sequence for the appropriate polypeptide. Thus, a promoter nucleotide sequence is operably linked to, e.g., the antibody heavy chain sequence if the promoter nucleotide sequence controls the transcription of the appropriate nucleotide sequence.
- [0076] In addition, sequences encoding appropriate signal peptides that are not naturally associated with antibody heavy and/or light chain sequences can be incorporated into expression vectors. For example, a nucleotide sequence for a signal peptide (secretory leader) may be fused in-frame to the polypeptide sequence so that the antibody is secreted to the periplasmic space or into the medium. A signal peptide that is functional in the intended host cells enhances extracellular secretion of the appropriate antibody. The signal peptide may be cleaved from the polypeptide upon secretion of antibody from the cell.
- [0077] The vector may be a plasmid vector, a single or double-stranded phage vector, or a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides by well known techniques for introducing DNA and RNA into cells. The vectors, in the case of phage and viral vectors also may be introduced into cells as packaged or encapsulated virus by well known techniques for infection and transduction. Viral vectors may be replication competent or replication defective. In the latter case viral propagation

generally will occur only in complementing host cells. Cell-free translation systems could also be employed to produce the protein using RNAs derived from the present DNA constructs.

- [0078] Prokaryotes useful as host cells in the present invention include gram negative or gram positive organisms such as *E. coli*, and *B. subtilis*. In a prokaryotic host cell, a polypeptide may include an N-terminal methionine (Met) residue to facilitate expression of the recombinant polypeptide in the prokaryotic host cell. The N-terminal Met may be cleaved from the expressed recombinant antibody polypeptide. Promoter sequences commonly used for recombinant prokaryotic host cell expression vectors include β -lactamase and the lactose promoter system.
- [0079] Expression vectors for use in prokaryotic host cells generally comprise one or more phenotypic selectable marker genes. A phenotypic selectable marker gene is, for example, a gene encoding a protein that confers antibiotic resistance or that supplies an autotrophic requirement. Examples of useful expression vectors for prokaryotic host cells include those derived from commercially available plasmids such as the cloning vector pBR322 (ATCC 37017). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells. To construct an expression vector using pBR322, an appropriate promoter and a DNA sequence are inserted into the pBR322 vector. Other commercially available vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden), pGEM1 (Promega Biotec, Madison, Wisconsin, USA), and the pET (Novagen, Madison, Wisconsin, USA) and pRSET (Invitrogen Corporation, Carlsbad, California, USA) series of vectors (Studier, F.W., J. Mol. Biol. 219: 37 (1991); Schoepfer, R. Gene 124: 83 (1993)).
- [0080] Promoter sequences commonly used for recombinant prokaryotic host cell expression vectors include T7, (Rosenberg, et al. Gene 56, 125-135 (1987)), β -lactamase (penicillinase), lactose promoter system (Chang et al., Nature 275:615, (1978); and Goeddel et al., Nature 281:544, (1979)), tryptophan (trp) promoter system (Goeddel et al., Nucl. Acids Res. 8:4057, (1980)), and tac promoter (Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) Yeasts useful as host cells in the present invention include those from the genus *Saccharomyces*, *Pichia*, *Actinomyces* and *Kluyveromyces*. Yeast vectors will often contain an origin of replication sequence from a 2 μ yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene. Suitable promoter sequences for yeast vectors include, among others, promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., J. Biol. Chem. 255:2073, (1980))

or other glycolytic enzymes (Holland et al., Biochem. 17:4900, (1978)) such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other suitable vectors and promoters for use in yeast expression are further described in Fleer et al., Gene, 107:285-195 (1991). Other suitable promoters and vectors for yeast and yeast transformation protocols are well known in the art.

- [0081] Yeast transformation protocols are known to those of skill in the art. One such protocol is described by Hinnen et al., Proc. Natl. Acad. Sci., 75:1929 (1978). The Hinnen protocol selects for Trp⁺ transformants in a selective medium, wherein the selective medium consists of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μ g/ml adenine, and 20 μ g/ml uracil.
- [0082] Mammalian or insect host cell culture systems well known in the art could also be employed to express recombinant antibodies, e.g., Baculovirus systems for production of heterologous proteins in insect cells (Luckow and Summers, Bio/Technology 6:47 (1988)) or NS0 or Chinese hamster ovary (CHO) cells for mammalian expression may be used. Transcriptional and translational control sequences for mammalian host cell expression vectors may be excised from viral genomes. Commonly used promoter sequences and enhancer sequences are derived from Polyoma virus, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus (CMV). DNA sequences derived from the SV40 viral genome may be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell, e.g., SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment which may also contain a viral origin of replication.
- [0083] Exemplary expression vectors for use in mammalian host cells are well known in the art. The immunogen IL13 polypeptide may, when beneficial, be expressed as a fusion protein that has the IL13 polypeptide attached to a fusion segment. The fusion segment often aids in protein purification, e.g., by permitting the fusion protein to be isolated and purified by affinity chromatography. Fusion proteins can be produced by culturing a recombinant cell transformed with a fusion nucleic acid sequence that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of the protein. Fusion segments may include, but are not limited to, immunoglobulin Fc regions, glutathione-S-transferase, β -galactosidase, a poly-histidine segment capable of binding to a divalent metal ion, and maltose binding protein.

POLYNUCLEOTIDES ENCODING ANTIBODIES

- [0084] The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody.
- [0085] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques* 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.
- [0086] Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A⁺ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.
- [0087] Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

- [0088] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the CDRs by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.
- [0089] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.
- [0090] Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., Science 242:1038-1041 (1988)).

METHODS OF PRODUCING ANTI-IL13 ANTIBODIES

- [0091] The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.
- [0092] Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody or a fragment of the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof, of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Pat. No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.
- [0093] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.
- [0094] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not

limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., Baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *E. coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

[0095] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0096] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody

coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0097] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

[0098] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, COS, 293, 3T3, or myeloma cells.

[0099] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be

allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[0100] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk, hgprt or aprt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Proc. Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, Biotherapy 3:87-95 (1991)); and hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

[0101] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, "The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells" (DNA Cloning, Vol.3. Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

[0102] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light

chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature* 322:52 (1986); Kohler, *Proc. Natl. Acad. Sci. USA* 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

- [0103] Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and size-exclusion chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.
- [0104] The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide. Fused or conjugated antibodies of the present invention may be used in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Pat. No. 5,474,981; Gillies et al., *Proc. Natl. Acad. Sci.* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452(1991), which are incorporated by reference in their entireties.
- [0105] Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)) and the "flag" tag.

DIAGNOSTIC USES FOR ANTI-IL13 ANTIBODIES

- [0106] The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by biotinylation, HRP, or any other detectable moiety.
- [0107] Antibodies of the present invention may be used, for example, but not limited to, to purify or detect IL13, including both in vitro and in vivo diagnostic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of IL13 in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).
- [0108] As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays.
- [0109] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of an allergic response as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Pat. No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine

fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{111}In or ^{99}Tc .

- [0110] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.
- [0111] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).
- [0112] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980, which is incorporated herein by reference in its entirety.
- [0113] Labeled antibodies, and derivatives and analogs thereof, which specifically bind to IL13 can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of IL13. The invention provides for the detection of aberrant expression of IL13, comprising (a) assaying the expression of IL13 in cells or body fluid of an individual using one or more antibodies of the present invention specific to IL13 and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed IL13 expression level compared to the standard expression level is indicative of aberrant expression.
- [0114] The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of IL13 in cells or body fluid of an individual using one or more antibodies of

the present invention and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed gene expression level compared to the standard expression level is indicative of a particular disorder.

- [0115] Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.
- [0116] One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of IL13 in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to IL13; b) waiting for a time interval following the administration permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of IL13. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.
- [0117] It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99}Tc . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo imaging is described in S. W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S. W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

- [0118] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.
- [0119] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.
- [0120] Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.
- [0121] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Pat. No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

DISEASE PREDISPOSITION DIAGNOSTICS

- [0122] In another aspect, the present invention provides a method for diagnosing the predisposition of a patient to develop diseases caused by the unregulated expression of cytokines. Increased amounts of IL13 in certain patient cells, tissues, or body fluids may indicate that the patient is predisposed to certain immune diseases. In one embodiment, the method comprises collecting a cell, tissue, or body fluid sample a subject known to have low or normal levels of IL13, analyzing the tissue or body fluid for the presence of IL13 in the tissue, and predicting the predisposition of the patient to certain immune diseases based upon the level of expression of IL13 in the tissue or body fluid. In another embodiment, the method comprises collecting a cell, tissue, or body fluid sample known to contain a defined level of IL13 from a

patient, analyzing the tissue or body fluid for the amount of IL13, and predicting the predisposition of the patient to certain immune diseases based upon the change in the amount of IL13 compared to a defined or tested level established for normal cell, tissue, or bodily fluid. The defined level of IL13 may be a known amount based upon literature values or may be determined in advance by measuring the amount in normal cell, tissue, or body fluids. Specifically, determination of IL13 levels in certain tissues or body fluids permits specific and early, preferably before disease occurs, detection of immune diseases in the patient. Immune diseases that can be diagnosed using the present method include, but are not limited to, the immune diseases described herein. In the preferred embodiment, the tissue or body fluid is peripheral blood, peripheral blood leukocytes, biopsy tissues such as lung or skin biopsies, and tissue.

THERAPEUTIC USES OF ANTI-IL13 ANTIBODIES

[0123] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) can be used as a therapeutic. The present invention is directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, for treating IL13-mediated diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention as described below (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of IL13, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of IL13 includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[0124] Anti-IL13 antibodies of the present invention may be used therapeutically in a variety of diseases. The antagonist antibodies may be used for the treatment of various immune diseases, including, but not limited to allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria; autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiform and contact dermatitis, psoriasis; rheumatoid arthritis, juvenile chronic arthritis; inflammatory bowel disease (i.e., ulcerative

colitis, Crohn's disease); idiopathic interstitial pneumonia; goblet cell metaplasia; inflammatory and fibrotic lung diseases such as cystic fibrosis, gluten-sensitive enteropathy, and Whipple's disease; immunologic diseases of the lung such as eosinophilic pneumonia, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis; chronic obstructive pulmonary disease, RSV infection, osteoporosis, and Hodgkin's lymphoma.

- [0125] Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for monitoring or therapeutic purposes without undue experimentation.
- [0126] The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 IL-7, IFN), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.
- [0127] The antibodies of the invention may be administered alone or in combination with other types of treatments, such as immunotherapy, bronchodilators, anti-IgE molecules, anti-histamines, or anti-leukotrienes. Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human or humanized antibodies, or active fragments thereof, are administered to a human patient for therapy or prophylaxis.
- [0128] It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against IL13, fragments or regions thereof, for therapy of disorders. Antibodies directed against IL13 are useful for inhibiting allergic reactions in animals. For example, by administering a therapeutically acceptable dose of an antibody, or antibodies, of the present invention, or a cocktail of the present antibodies, or in combination with other antibodies of varying sources, the animal may not elicit an allergic response to antigens.
- [0129] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of the antibody or pharmaceutical composition of the invention. In a preferred aspect, the antibody is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, non-human primates etc., and is preferably a mammal, including humans.

- [0130] Various delivery systems are known and can be used to administer an antibody of the present invention, including injection, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu et al., J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc.
- [0131] The anti-IL13 antibody can be administered to the mammal in any acceptable manner. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, inhalation and oral routes. The antibodies or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the therapeutic antibodies or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. The antibody may also be administered into the lungs of a patient in the form of a dry powder composition (See e.g., U.S. Pat. No. 6,514,496).
- [0132] In a specific embodiment, it may be desirable to administer the therapeutic antibodies or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, topical application, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering an antibody of the invention, care must be taken to use materials to which the protein does not absorb.
- [0133] In another embodiment, the antibody can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*).
- [0134] In yet another embodiment, the antibody can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med.

321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target.

[0135] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of the antibody, and a physiologically acceptable carrier. In a specific embodiment, the term "physiologically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such physiological carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain an effective amount of the antibody, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0136] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic

aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0137] In another aspect, the present invention provides a method for preventing or treating IL13-mediated diseases in a mammal. The method comprises administering a disease preventing or treating amount of anti-IL13 antibody to the mammal. The anti-IL13 antibody binds to IL13 and regulates cytokine and cellular receptor expression resulting in cytokine levels characteristic of non-disease states. Preferably, the disease is an allergy, asthma, autoimmune, or other inflammatory disease. Most preferably, the disease is an allergy or asthma.

[0138] The amount of the antibody which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of IL13 can be determined by standard clinical techniques. The antibody can be administered in treatment regimes consistent with the disease, e.g., a single or a few doses over one to several days to ameliorate a disease state or periodic doses over an extended time to prevent allergy or asthma. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0139] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the

invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

- [0140] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.
- [0141] IL13 is also believed to be useful in the treatment of other diseases, such as some cancers like B-cell chronic lymphocytic leukemia and Hodgkin's disease by the modulation of apoptosis or tumor cell growth (Kapp et al., J. Exp. Med., 189: 1939 (1999), Skinnider et al., Leuk. Lymphoma, 43: 1203 (2002)). Inhibitors of IL13 may also be effective as cancer immunotherapeutics by boosting type-1 associated anti-tumor defenses.
- [0142] In addition, anti-IL13 antibodies may be used in treating respiratory syncytial virus (RSV) infection. The development of childhood asthma is influenced by several factors, including environmental and infectious stimuli. Respiratory syncytial virus can exacerbate and promote severe asthmatic-type responses and the preferential production of IL13 during primary RSV infection appears to play a role (Lukacs et al., J. Immunol. 167:1060-1065 (2001)). Moreover, infection of the airways with RSV can induce IL13-dependent changes in airway function and promotes an environment contributing to the development of severe allergic asthmatic responses. Inhibition of IL13 with anti-IL13 antibodies may reduce this change in airway function and lessen the effects of RSV infection in asthmatics.
- [0143] In addition, the antibodies of the present invention may be conjugated to various effector molecules such as heterologous polypeptides, drugs, radionucleotides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 396,387. An antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ²¹³Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologues thereof. Therapeutic agents include,

but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

- [0144] The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- α , TNF- β , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi et al., Int. Immunol., 6:1567-1574 (1994)), VEGF (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

ANTIBODY-BASED GENE THERAPY

- [0145] In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of IL13, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.
- [0146] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.
- [0147] For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan

and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

- [0148] In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.
- [0149] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.
- [0150] In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Pat. No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which

the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

[0151] In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates the delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdrl gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. Gen. and Dev. 3:110-114 (1993).

[0152] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Curr. Opin. Gen. Dev. 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143-155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

[0153] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Pat. Nos. 5,436,146; 6,632,670; 6,642,051).

- [0154] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.
- [0155] In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.
- [0156] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.
- [0157] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.
- [0158] In a preferred embodiment, the cell used for gene therapy is autologous to the patient. In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect.

In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

[0159] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription

THERAPEUTIC AND PROPHYLACTIC ACTIVITY

[0160] The antibodies or compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition includes, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

EXAMPLES

EXAMPLE 1

Preparation of Mutated, Inactive Human IL13/Fc (MT-IL13/Fc)

A. Cloning And Construction Of An Expression Plasmid For MT-IL13/Fc

[0161] It was reported that human IL13 with a mutation (glutamic acid to lysine) at amino acid residue #13 bound IL13R α 1 with equal or higher affinity but had lost the ability to activate IL13R α 1-bearing cells (Thompson et al., J. Biol. Chem., 274: 29944 (1999)). This mutated, inactive IL13, designated MT-IL13, was expressed in human embryonic kidney cells 293-T. The purified recombinant protein was used as the immunogen in the present invention to generate anti-IL13 monoclonal antibodies.

[0162] Two oligonucleotide primers: 5' AAGCTTTCCCCAGGCCCTGTGCCTCCCTCTACAGCCCTCAGGAAGCTCAT3' (SEQ ID NO 9) and 5' CTCGAGGTTGAACCGTCCCTCGCGAAAAAG 3' (SEQ ID NO 10) corresponding to the oligonucleotide sequence of MT-IL13 gene were synthesized and used as templates in polymerase chain reactions (PCR) to clone the IL13 gene from human testis cDNA library (BD Biosciences Clontech, Palo Alto, CA). The PCR fragment (342 base pairs) which lacked the predicted signal peptide sequence of IL13 was ligated into the pSecTag/FRT vector (Invitrogen, Carlsbad, CA) that contained a secretion signal peptide sequence at the 5' end and a human Fcγ1 (hinge and constant regions CH2 and CH3) sequence at the 3' end. The construct's composition was confirmed by sequencing.

B. Production of MT-IL13/Fc from Transfected 293T Cells

[0163] For transient expression of MT-IL13/Fc, purified plasmid DNA was transfected into 293T cells by Lipofectamine 2000 (Invitrogen), according to the manufacturer's protocol. At 72 hours post-transfection, culture supernatants from transfected cells were collected for purification. For stable expression of MT-IL13/Fc, cell lines were established using a Flp-In 293T cell line (Invitrogen). To confirm expression, culture supernatants were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were transferred to nitrocellulose membrane and detected by reaction with horseradish peroxidase (HRP) conjugated mouse anti-human IgG (Fc) monoclonal antibody (Sigma, St. Louis, MO) or polyclonal goat anti-IL13 antibodies (R&D Systems, Minneapolis, MN), which were then detected with HRP-donkey anti-goat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). The immunoreactive proteins were identified on film, using enhanced chemiluminescence detection (Supersignal West Pico Chemiluminescent Substrate, Pierce, Rockford, IL).

C. Purification of MTIL13/Fc

[0164] MT-IL13/Fc was purified with a hyper-D protein A affinity column (Invitrogen) equilibrated with phosphate-buffered saline (PBS). After applying the cell culture supernatant to the column, the resin was washed with more than 20 column volumes of PBS. Then, the resin was washed with SCC buffer (0.05 M sodium citrate, 0.5 M sodium chloride, pH 6.0) to remove unbound proteins. The IL13 fusion proteins were then eluted (0.05 M sodium citrate, 0.15 M sodium chloride, pH 3.0) and dialyzed in PBS.

[0165] Fractions from the affinity column containing MT-IL13/Fc were analyzed by SDS-PAGE. The purity of the proteins were analyzed by Coomassie Blue staining and the identity of the proteins by Western immunoblotting using goat anti-human IgG (Fc) antibody (Sigma) and goat anti-human IL13 antibody (R&D Systems) as described above.

EXAMPLE 2

Generation Of Anti-IL13 Monoclonal Antibodies

[0166] Male A/J mice (Harlan, Indianapolis, IN), 8-12 weeks old, were injected subcutaneously with 20 μ g of either MT-IL13/Fc in complete Freund's adjuvant (Difco Laboratories, Detroit, MI) in 200 μ L of PBS pH 7.4. At two-week intervals the mice were twice injected subcutaneously with 20 μ g of the IL13 immunogen in incomplete Freund's adjuvant. Then, two weeks later and three days prior to sacrifice, the mice were again injected intraperitoneally with 20 μ g of the same immunogen in PBS. Spleen cells isolated from one or more than one antigen-immunized mouse were used for fusion. Similar procedures of immunization and fusion were also used with *E. coli* expressed human IL13 (R&D Systems) as immunogen.

[0167] In the fusion leading to the generation of the anti-IL13 mAb 228B/C-1, 26.4×10^6 spleen cells and 58.8×10^6 spleen cells from two immunized mice were combined. For each fusion, single cell suspensions were prepared from the spleen of immunized mice and used for fusion with Sp2/0 myeloma cells. Sp2/0 and spleen cells at a ratio of 1:1 were fused in a medium containing 50% polyethylene glycol (M.W. 1450) (Kodak, Rochester, NY) and 5% dimethylsulfoxide (Sigma). The cells were then adjusted to a concentration of 1.5×10^5 spleen cells per 250 μ L of the suspension in DMEM medium (Invitrogen, CA), supplemented with 10% fetal bovine serum, 100 units/mL of penicillin, 100 μ g/mL of streptomycin, 0.1 mM hypoxanthine, 0.4 μ M aminopterin, and 16 μ M thymidine. Two hundred and fifty microliters of the cell suspension were added to each well of about fifty 96-well microculture plates. After about ten days culture supernatants were withdrawn for screening for reactivity with MT-IL13/Fc in ELISA.

[0168] Wells of Immulon 2 (Dynatech Laboratories, Chantilly, VA) microtest plates were coated by adding purified MT-IL13/Fc (0.1 μ g/mL) overnight at room temperature. After the coating solution was removed by flicking of the plate, 200 μ L of a blocking/diluting buffer (PBS containing 2 % bovine serum albumin and 0.05 % TWEEN® 20) was added to each well for one hour to block the non-specific sites. One hour later, the wells were then washed with PBST buffer (PBS containing 0.05% TWEEN® 20). Fifty microliters of culture supernatant was collected from each fusion well, mixed with 50 μ L of the blocking/diluting buffer and then

added to the individual wells of the microtest plates. After one hour of incubation, the wells were washed with PBST. The bound murine antibodies were then detected by reaction with HRP-conjugated goat anti-mouse IgG (Fc specific) (Jackson ImmunoResearch Lab, West Grove, PA) and diluted at 1:2,000 with the blocking/diluting buffer. Peroxidase substrate solution containing 0.1% 3,3',5,5' tetramethyl benzidine (Sigma, St. Louis, MO) and 0.003% hydrogen peroxide (Sigma) was added to the wells for color development for 30 minutes. The reaction was terminated by the addition of 50 μ L of 2 M H_2SO_4 per well. The OD_{450} of the reaction mixture was measured with a BioTek ELISA Reader (BioTek Instruments, Winooski, VM).

- [0169] The culture supernatants from the positive wells of MT-IL13/Fc screening were then tested for negative binding to an irrelevant Fy1 fusion protein. Final positive wells were then selected for single-cell cloning by limiting dilution. Culture supernatants from monoclonal antibodies were re-tested to confirm their reactivity by ELISA. Selected hybridomas were grown in spinner flasks and the spent culture supernatant collected for antibody purification by protein A affinity chromatography.
- [0170] The purified antibodies were tested by four assays: i) Cross-reactivity with 293T cell expressed MT-IL13/Fc and E. coli expressed mouse IL13; ii) Inhibition of IL13-autocrine proliferation of HDLM-2 and L-1236 cells; iii) Inhibition of IL13-induced STAT6 phosphorylation in THP-1 cells; and iv) Inhibition of IL13-regulated CD14 and CD23 expression on human monocytes.
- [0171] Seventy-three anti-IL13 mAbs were obtained from the fusions performed on MT-IL13/Fc and IL13 immunized mice. Thirty-nine of these mAbs were purified for characterization by ELISA and cell-based assays. Thirteen of these 39 mAbs inhibited autocrine IL13-induced proliferation of HDLM-2 and L-1236 cells (see assay description and results in Example 5). Four of the mAbs were found to be very strongly reactive with human IL13 in ELISA and were neutralizing against human IL13 in functional cell-based assays. These mAbs were designated 228B/C-1, 228A-4, 227-26, and 227-43. These antibodies were all generated using the glycosylated MT-IL13/Fc as immunogen.

EXAMPLE 3:

Reactivity Of Anti-IL13 Monoclonal Antibodies With Human And Mouse IL13 In ELISA

- [0172] The reactivity of various anti-IL13 monoclonal antibodies was tested by ELISA. Different wells of 96-well microtest plates were coated with either E. coli expressed non-glycosylated human IL13 (R&D Systems), 293T cell expressed glycosylated MT-IL13/Fc, or E. coli expressed mouse IL13 (R&D Systems) by the addition of 100 μ L of IL13 protein at 0.1 μ g/mL in PBS. After overnight incubation at room temperature, the wells were treated with PBSTB (PBST containing 2% BSA) to saturate the remaining binding sites. The wells were then washed with PBST.
- [0173] One hundred microliters of two-fold serially diluted anti-IL13 mAbs (0.5 μ g/mL (3.33 nM) to 0.05 ng/mL (0.00033 nM)) were added to the wells for 1 hour at room temperature. An anti-IL13 mAb JES-5A2 from (BD Biosciences-Pharmingen, San Diego, CA) was also tested as a positive control. This antibody was generated by using E. coli expressed human IL13 as immunogen. An isotype-matched mouse anti-HIV-1 gp120 mAb was used as irrelevant negative control. The wells were then washed with PBST. Bound antibody was detected by incubation with diluted HRP-goat anti-mouse IgG (Fc) (Jackson ImmunoResearch) for 1 hour at room temperature. Peroxidase substrate solution was then added for color development as described above. The OD₄₅₀ was measured using an ELISA reader.
- [0174] Fig. 1 shows the dose-dependent binding of anti-IL13 mAbs 228B/C-1, 228A-4, 227-26, 227-43, and the negative control in ELISA. Among these mAbs, 228B/C-1 showed the strongest reactivity. Fig. 2 shows the dose-dependent binding of the anti-IL13 mAbs to MT-IL13/Fc in ELISA. 228B/C-1 and 228A-4 showed the strongest reactivity with MT-IL13/Fc, whereas 227-26 and 227-43 showed moderate reactivity.
- [0175] Figs 1 and 2 show that 228B/C-1 has highest affinity for both glycosylated and non-glycosylated human IL13 among all the anti-IL13 mAbs tested. All these anti-IL13 mAbs did not cross-react with mouse IL13 in ELISA (data no shown).

EXAMPLE 4

Lack Of Competition Of 228B/C-1-Hrp Binding To Human IL13 By JES10-5A2

- [0176] To address whether JES10-5A2 and 228B/C-1 bind to the same epitope on human IL13, a competition ELISA was used to examine the effect of JES10-5A2 on 228B/C-1-HRP binding to E. coli expressed human IL13. Each well of 96-well microtest plates were incubated with 100 μ L of IL13 protein at 0.1 μ g/mL in PBS. After overnight incubation at room temperature, the wells were treated with PBSTB (PBST containing 2% BSA) to saturate the remaining binding sites. The wells were then washed with PBST. Fifty microliters of two fold serially diluted 228B/C-1 and JES10-5A2 (from a final concentration of 20 μ g/mL to 9.76 ng/mL) were mixed with 50 μ L of pre-titrated 228B/C-1-HRP (at 1:6,400 dilution). The mixtures were then added to the wells and incubated for 1 hour at room temperature. Peroxidase substrate solution was then added for color development as described above. The OD₄₅₀ was measured using an ELISA reader.
- [0177] Fig. 3 demonstrates that JES10-5A2 does not compete with the binding of 228B/C-1-HRP to human IL13, indicating that 228B/C-1 and JES10-5A2 bind to different sites on human IL13.

EXAMPLE 5

Screening Assay For Anti-IL13 Neutralizing Monoclonal Antibodies Using L-1236 And HdIm-2 Cells

- [0178] L-1236 and HDLM-2 are Hodgkin lymphoma cell lines obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). These cell lines produce IL13 which in turn activates their cell proliferation in an autocrine fashion (Kapp U et.al., J. Exp. Med. 189:1939 (1999)).
- [0179] Cells were cultured (25,000 cells/well) in the presence or absence of different anti-IL13 mAb (0.2, 0.02 and 0.002 μ g/mL) in 5% CO₂ at 37°C for 3-5 days. Cell proliferation was then measured either by an assay using the tetrazolium compound MTS (Promega, Madison, WI) (readouts at OD₄₉₀) or by the incorporation of ³H-thymidine (Amersham Biosciences, Piscataway, NJ).
- [0180] The addition of an anti-IL13 neutralizing mAb to the culture of these cell lines was expected to inhibit their proliferation by the binding and inactivation of the IL13 produced by these cells.

The results illustrated in Figure 4 shows the effect of anti-IL13 mAb of the present invention on the proliferation of L-1235 cells. mAb 228B/C-1 displays the highest potency of inhibition of L-1236 cell proliferation in a dose-dependent manner among the neutralizing antibodies tested. TA1-37 (an anti-IL13 mAb generated by using E. coli expressed human IL13 as immunogen) did not have any inhibitory activity even at a dose as high as 0.2 $\mu\text{g/mL}$. Similar results were obtained with HDLM-2 cells.

EXAMPLE 6

Assay For IL13-Regulated CD14 And CD23 Expression On Primary Human Monocytes

- [0181] IL13 induces suppression of CD14 expression and the up-regulation of CD23 expression in the human monocytes (de Waal Malefyt et al., J. Immunol., 151: 6370 (1993), Chomarat et al., Int. Rev. Immunol., 17: 1 (1998)). Peripheral blood leukocytes (PBLs) were isolated from freshly collected, heparinized whole blood of healthy human donors by density-gradient centrifugation in Histopaque-1077 (Sigma). PBLs (1.5×10^6) suspended in RPMI-1640 medium (Invitrogen) with 5% fetal bovine serum were added to each well of a 96-well tissue culture plate containing recombinant IL13 (final 10 ng/mL = 0.813 nM) and an anti-IL13 monoclonal antibody or an irrelevant antibody (three-fold serial dilutions, from a final 12 $\mu\text{g/mL}$ = 80 nM). CD14 expression or CD23 expression on monocytes was suppressed or up-regulated, respectively, by the addition of 0.813 nM human IL13 to the incubating medium. The medium control contained RPMI-1640/FBS medium without recombinant IL13.
- [0182] The cells were incubated in 5% CO₂ at 37°C for 2 days. The cells were harvested for staining with anti-CD14-FITC or anti-CD23-PE (BD Biosciences-Pharmingen). The expression levels of CD14 and CD23 in the monocyte population were measured by flow cytometry and represented by Median Fluorescence Intensity (MFI).
- [0183] The effects of anti-IL13 mAbs on IL13-suppressed CD14 expression on human monocytes are depicted in Fig. 5. Among all the anti-IL13 mAbs tested, 228B/C-1 had the highest potency in inhibiting the effect of IL13 on CD14 expression. Complete inhibition of the effect of IL13 was achieved at 0.33 nM. The inhibitory activities of mAbs 227-26 and 228A-4 were moderate, whereas that of JES10-5A2 was weak. The effect of IL13 could not be completely inhibited by JES10-5A2 even at 80nM.
- [0184] The effects of anti-IL13 mAbs on IL13-induced CD23 up-regulation on human monocytes are depicted in Fig. 6. Similar to the results on CD14 expression (Fig. 5), 228B/C-1 was most

potent in inhibiting the effect of IL13 on CD23 expression among the anti-IL13 mAbs tested. Complete inhibition by 228B/C-1 was achieved at 0.33 nM. The inhibitory potency of JES10-5A2 was weak.

[0185] Based on the results presented in Figs. 5 and 6, complete inhibition of IL13 by 228B/C-1 can be achieved at a molar stoichiometric ratio of 1:2 (mAb:IL13), and, therefore, 228B/C-1 is a very high affinity neutralizing mAb against human IL13.

EXAMPLE 7

IL13-Induced STAT6 Phosphorylation Assay In THP-1 Cells

[0186] IL13 can activate the myeloid cell line THP-1(ATCC, Manassas, VA) to induce phosphorylation of STAT6 which is a critical step in the signal transduction pathway of IL13 (Murata T et al., Int. Immunol. 10: 1103-1110 (1998). The anti-IL13 mAbs were tested for inhibition of IL13 in this assay. THP-1 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen) supplemented with 5 % fetal bovine serum. On the day of experiments, the cells were washed and incubated in serum-free DMEM at 37°C in 5% CO₂ for 2 hours. 0.3×10^6 cells in 80 μ L of the serum-free medium were then added to each well of a 96-well round-bottom plate. One hundred and twenty microliters of medium containing human IL13 (final concentration of 10 ng/mL = 0.813 nM) and anti-IL13 mAbs (5 fold serial dilutions, from final concentration of 0.5 μ g/mL = 3.333 nM). Negative control wells containing either no IL13 or IL13 and an isotype-matched irrelevant mouse mAb. The mixtures were then incubated at 37°C under 5% CO₂ for 10 min. The plates were then centrifuged at 300 x g for 3 minutes at 4°C. After discarding the supernatant, the cell pellets were resuspended in 100 μ L of Laemmli non-reducing sample buffer (SDS-PAGE loading buffer, BioRad, CA) and then transferred to microcentrifuge tubes. The tubes were heated at 95 °C for 5 minutes and then centrifuged at 10,000 x g for 10 minutes at room temperature. The supernatants were collected and analyzed by 4-20% gradient SDS-PAGE. The separated proteins were transferred to PVDF membrane which was then incubated with diluted mouse anti-human Stat6 (Y641, phospho-specific) mAb (BD Biosciences Pharmingen). The bound antibody was detected by HRP conjugated goat anti-mouse IgG (Fc) antibodies (Jackson ImmunoResearch Laboratories). The immunoreactive proteins were identified on film, using enhanced chemiluminescence detection (Supersignal West Pico Chemiluminescent Substrate, Pierce)

[0187] Fig. 7 depicts the results of the effect of anti-IL13 mAbs on IL13-induced phosphorylation of Stat6 in THP-1 cells. Stat6 is phosphorylated in THP-1 cells treated with 0.813 nM human IL13. Dose-dependent inhibition of Stat6 phosphorylation was found when the cells were

treated with mAbs 228B/C-1, 228A-4, 227-26, 227-43 and JES10-5A2. mAb 228B/C-1 is the most potent neutralizing antibodies among the anti-IL13 mAbs tested. Complete inhibition by 228B/C-1 was achieved at a concentration between 0.667 nM and 0.133 nM. The approximate molar stoichiometric ratio between 228B/C-1 and IL13 for complete inhibition was 1:2. It is consistent with the data shown in Figs. 5 and 6.

EXAMPLE 8

Molecular Cloning Of Heavy And Light Chain Genes Encoding Anti-IL13 Monoclonal Antibodies

- [0188] Total RNA was isolated from hybridoma cells using a QIAGEN kit (Valencia, CA). Reverse transcription (first strand cDNA) reaction was carried out as follows: 1-1.5 mg of total RNA was mixed with 1 ml 10 mM dNTPs, 50 ng random Hexamers, and RNase free water in a final volume of 12 mL.
- [0189] The reaction mixture was incubated at 65°C for 5 minutes and placed on ice immediately for 1 minute. After a brief centrifugation, the following reagents were added: 4 mL of 5X first strand buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM MgCl₂), 2 mL of 0.1 mM DTT, and 1 mL of RNaseOUT RNase inhibitor (40 U/mL). After mixing, the reaction was incubated at room temperature for 2 minutes. One milliliter of Superscript II RT (50 U/ml) was then added to the mixture for incubation at 25°C for 10 minutes followed by 50 minutes at 42°C. After a brief centrifugation, the reaction was incubated for 15 minutes at 70°C to inactivate the reverse transcriptase. One microliter of RNase H (2 U/ml) was then added and the reaction was incubated for 20 minutes at 37°C to destroy RNA.
- [0190] To amplify the variable regions of the heavy and light chains, a method described by O'Brien and Jones (O'Brien S. and Jones T., "Humanizing antibodies by CDR grafting", Antibody Engineering, Springer Lab manual, Eds. Kontermann and Duble, S (2001)) was used. Briefly, 5' primers were selected from the signal peptide region (11 sets for light chain and 12 sets of degenerate primers for heavy chain) and 3' primers were selected from the constant region of either the light or heavy chain. 5' and 3' primers (1.5 mL of 10 mM) were mixed with 5 mL of 10X PCR buffer (250 mM Tris-HCl, pH 8.8, 20 mM MgSO₄, 100 mM KCl, 100 mM (NH₄)₂ SO₄, 1% Triton X-100, 1 mg/mL nuclease free BSA), 1 mL cDNA as prepared previously, 1 mL of Turbo pfu (Stratagene) and water to adjust the total volume of the reaction to 50 mL. PCR was performed as follows: 1 cycle at 94°C for 4 minutes; 25 cycles at 94°C for 30 seconds, at 53°C for 30 seconds, and at 72°C for 45 seconds; and 1 cycle at 72°C for 7 minutes. Reaction mixtures were resolved by electrophoresis in a 1% agarose gel.

[0191] Amplified DNA fragment was purified and cloned into a pcDNA3.1 vector. Cloning was carried out using the Invitrogen TOPO cloning kit following the manufacturer's suggested protocol (Invitrogen). Fifteen to twenty colonies of transformed E.coli were used for plasmid purification. Plasmids were sequenced using a T7 primer. The predominant sequences for the heavy and light chains were cloned into an M13 Fab expression vector by hybridization mutagenesis (Glaser S. et al. Antibody Engineering (Oxford University Press, New York(1995)), Near RI, BioTechniques 12: 88 (1992)). Binding properties of the expressed Fab were confirmed by ELISA. Figures 8-10 depict the VH and VL chain amino acid sequences for 228B/C, 228A-4, and 227-26, respectively.

Claims

[c1]

An antibody that binds specifically and with high affinity to both glycosylated and non-glycosylated human IL13, does not bind mouse IL13, and neutralizes human IL13 activity at an approximate molar ratio of 1:2 (mAb:IL13).

[c2]

The antibody of claim 1, wherein the antibody is 228B/C-1,

[c3]

The antibody of claim 1, wherein the antibody is 228A-4.

[c4]

The antibody of claim 1, wherein the antibody is 227-26.

[c5]

The antibody of claim 1, wherein the antibody is 227-43.

[c6]

An antibody, wherein the antibody comprises antigen binding regions derived from the light and heavy chain variable regions of an antibody of claim 1.

[c7]

The antibody of claim 6, wherein said antibody has a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 3, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 4.

[c8]

The antibody of claim 6, wherein said antibody has a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 5, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 6.

[c9]

The antibody of claim 6, wherein said antibody has a VL sequence at least 95% homologous to that set forth in SEQ ID NO: 7, and a VH sequence at least 95% homologous to that set forth in SEQ ID NO: 8.

[c10]

A hybridoma cell line that produces a monoclonal antibody selected from the group consisting of 228B/C-1, 228A-4, 227-26, and 227-43 and designated with the ATCC deposit number PTA-5657, PTA-5656, PTA-5654, and PTA-5655, respectively.

[c11]

A cell line comprising a nucleic acid encoding the antibody of claim 1.

[c12]

A vector comprising a nucleic acid encoding the antibody of claim 1.

[c13]

The antibody of claim 1, wherein the antibody is a monoclonal antibody.

[c14]

The antibody of claim 13, wherein the monoclonal antibody is a human antibody, a chimeric antibody, or a humanized antibody.

[c15]

A composition comprising the antibody of claim 1 and a physiologically acceptable carrier, diluent, excipient, or stabilizer.

[c16]

A method of treating a subject suffering from asthmatic symptoms comprising administering an amount of an antibody according to claim 1 effective to reduce the asthmatic symptoms.

[c17]

A method of claim 16 wherein the antibody down-regulates the activity of IL 13 in the patient.

[c18]

A method of claim 16, wherein the antibody reduces bronchial hyperresponsiveness in the patient.

[c19]

A method of claim 16, wherein the antibody reduces eosinophilia in the lungs of the subject.

[c20]

The method according to claim 16, wherein the antibody is administered by one or more of the routes selected from the group consisting of intravenous, intraperitoneal, inhalation, intramuscular, subcutaneous and oral.

[c21]

The method according to claim 20, wherein the antibody is administered by inhalation.

[c22]

An inhalation device that delivers to a patient a therapeutically effective amount of an antibody according to claim 1.

[c23]

A method of inhibiting the infection of respiratory syncytial virus (RSV) comprising administering an inhibiting amount of the antibody of claim 1.

[c24]

A method for detecting interleukin-13 protein in a patient suffering from an allergic disease, comprising the step of: allowing the antibody of claim 1 to contact a sample; and detecting the interleukin-13 through the occurrence of immunoreaction.

[c25]

A method for diagnosing overexpression of IL13 in a subject, comprising the steps of: (a) obtaining a sample from the subject; (b) combining the sample with an antibody according to claim 1 under conditions which would allow immunoreaction with IL13; and (c) determining whether or not IL13 is overexpressed relative to a normal level of expression of IL13.

[c26]

A method of producing the antibody of claim 1, comprising the steps of: a) producing an immunogenic compound comprising a glycosylated IL13 moiety and an immunogenic moiety; b) preparing an injectable solution comprising said immunogenic compound in phosphate buffered saline (PBS) and an adjuvant; c) immunizing a mouse with said injectable solution by a combination of intravenous and intraperitoneal injections, d) producing a hybridoma by fusing a spleen cell from said immunized mouse with a myeloma cell; e) selecting a hybridoma producing an antibody having the characteristics of the antibody of claim 1; and f) isolating said antibody.

[c27]

A recombinant antibody molecule, or an IL13-binding fragment thereof, comprising: at least one antibody heavy chain, or an IL13-binding fragment thereof, comprising non-human CDRs at positions 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3) (Kabat numbering) from a mouse anti-IL13 antibody, wherein positions 27-30 have the amino acid Gly 26, Phe 27, Ser 28, Leu 29, Asn 30, (SEQ ID NO: 18); and at least one antibody light chain, or an IL-13-binding fragment thereof, comprising non-human CDRs at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3) from a mouse anti-IL13 antibody, and framework regions from a monoclonal antibody.

[c28]

A DNA sequence encoding the antibody of claim 27.

[c29]

A vector comprising the DNA sequence of claim 28.

[c30]

A host cell comprising the vector of claim 29.

[c31]

A chimeric immunoglobulin chain comprising at least part of a human immunoglobulin constant region and at least part of a nonhuman immunoglobulin variable region having specificity to human IL13 .

[c32]

A method for inhibiting IgE antibody production in a patient, which comprises administering to the patient an IgE antibody production inhibiting effective amount of an antibody according to claim 1.

[c33]

The method according to claim 32, wherein the inhibition of IgE antibody production is intended to prevent bronchial asthma, to prevent allergic rhinitis, to prevent allergic dermatitis, to treat bronchial asthma, to treat allergic rhinitis, to treat urticaria, to prevent anaphylaxis, or to treat atopic dermatitis.

[c34]

A method of treating an IL13-mediated disorder in a patient, comprising administering to the patient an effective amount of an antibody or antigen-binding fragment thereof according to claim 1, wherein said antibody or antigen-binding fragment thereof inhibits binding of IL13 to its receptor and inhibits one or more functions associated with binding of the interleukin to said receptor.

[c35]

A method of treating an IgE-mediated disorder in a patient, comprising administering to the patient an effective amount of an antibody or antigen-binding fragment thereof according to claim 1, wherein said antibody or antigen-binding fragment thereof inhibits binding of IL13 to its receptor and inhibits one or more functions associated with binding of the interleukin to said receptor.

[c36]

A method for reducing the severity of asthma in a mammal comprising administering to the mammal a therapeutically effective amount of an anti-IL13 monoclonal antibody having the following characteristics; ability to bind human IL13 with a K_D between about 1×10^9 to about 1×10^{12} M, ability to inhibit one or more functions associated with binding of the interleukin IL13 to the IL13 receptor; and the antibody does not bind mouse IL13.

[c37]

The method of claim 36, wherein the anti-IL13 antibody is administered by inhalation, systemically, bolus injection, or continuous infusion.

Abstract of Disclosure

[0192] The present invention relates to high affinity anti-IL13 antibodies and their use in the treatment of allergic disease, including asthma, as well as the treatment of RSV infection, and osteoporosis.

Fig. 1 Binding of anti-IL13 mAbs to human IL-13 in ELISA

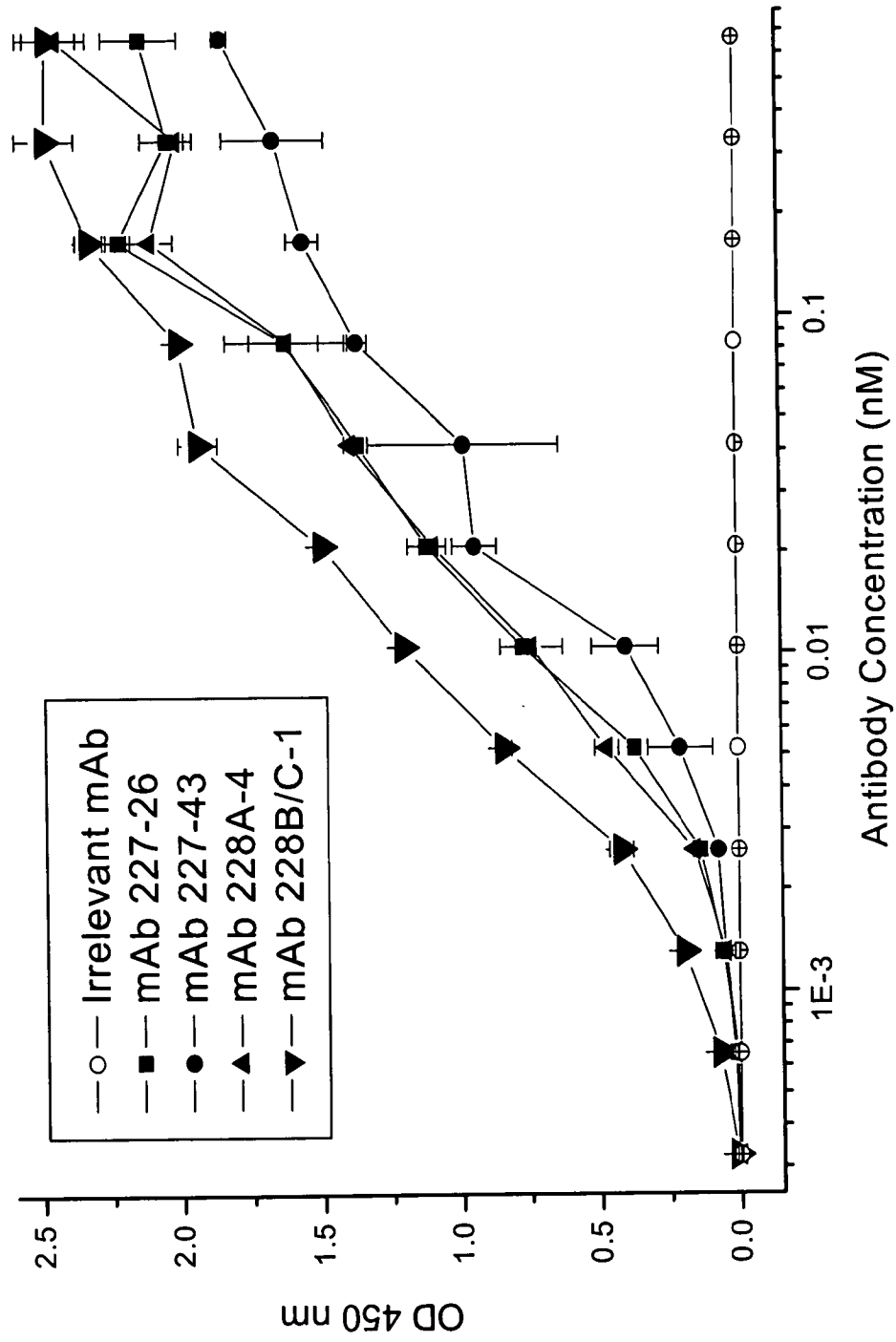


Fig. 2 Binding of anti-IL13 mAbs to MT-IL13/Fc

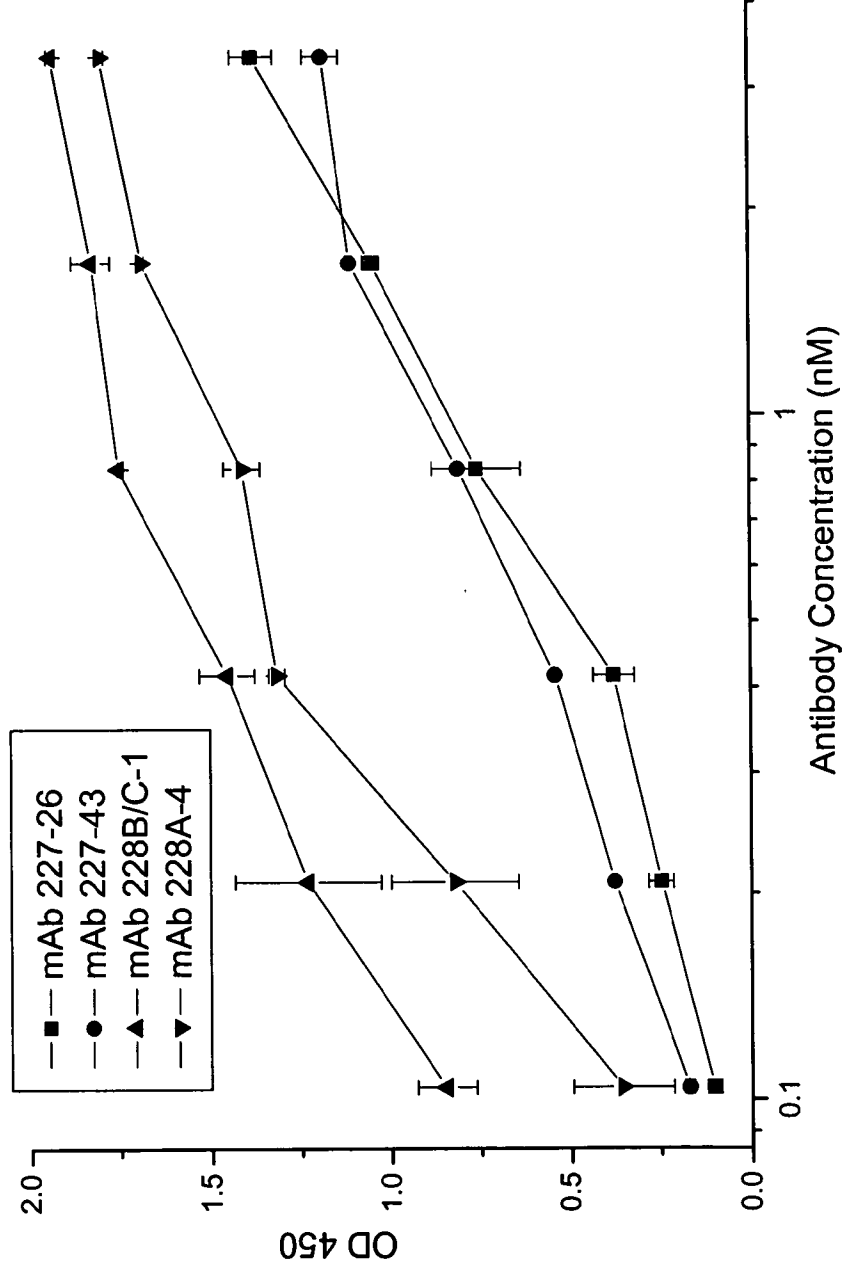


Fig. 3 Anti-IL13 mAb JES10-5A2 does not compete with the binding of mAb 228B/C-1-HRP to Human IL-13 in ELISA

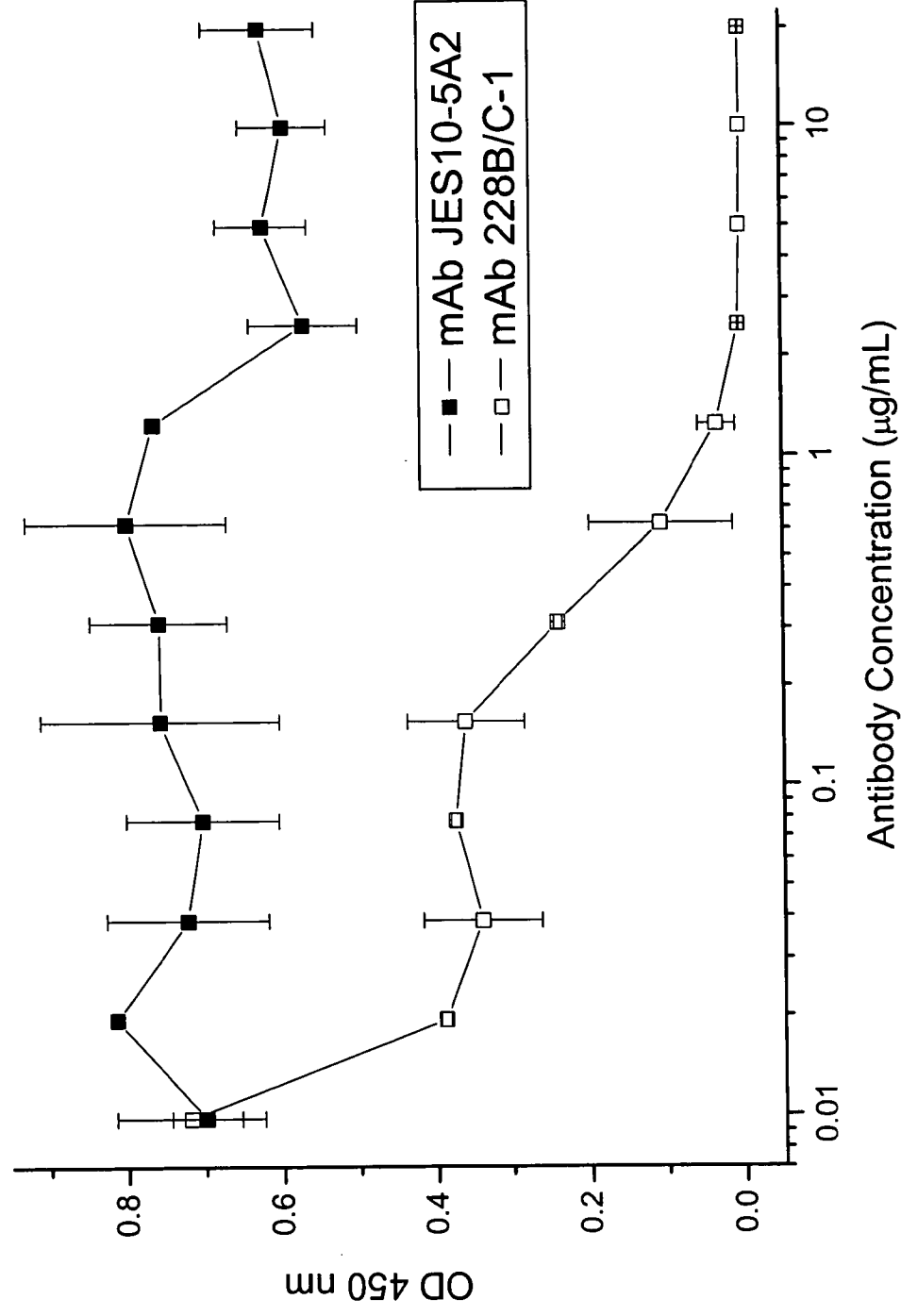


Fig. 4 Effect of anti-IL13 mAbs on the proliferation of L-1236 cells

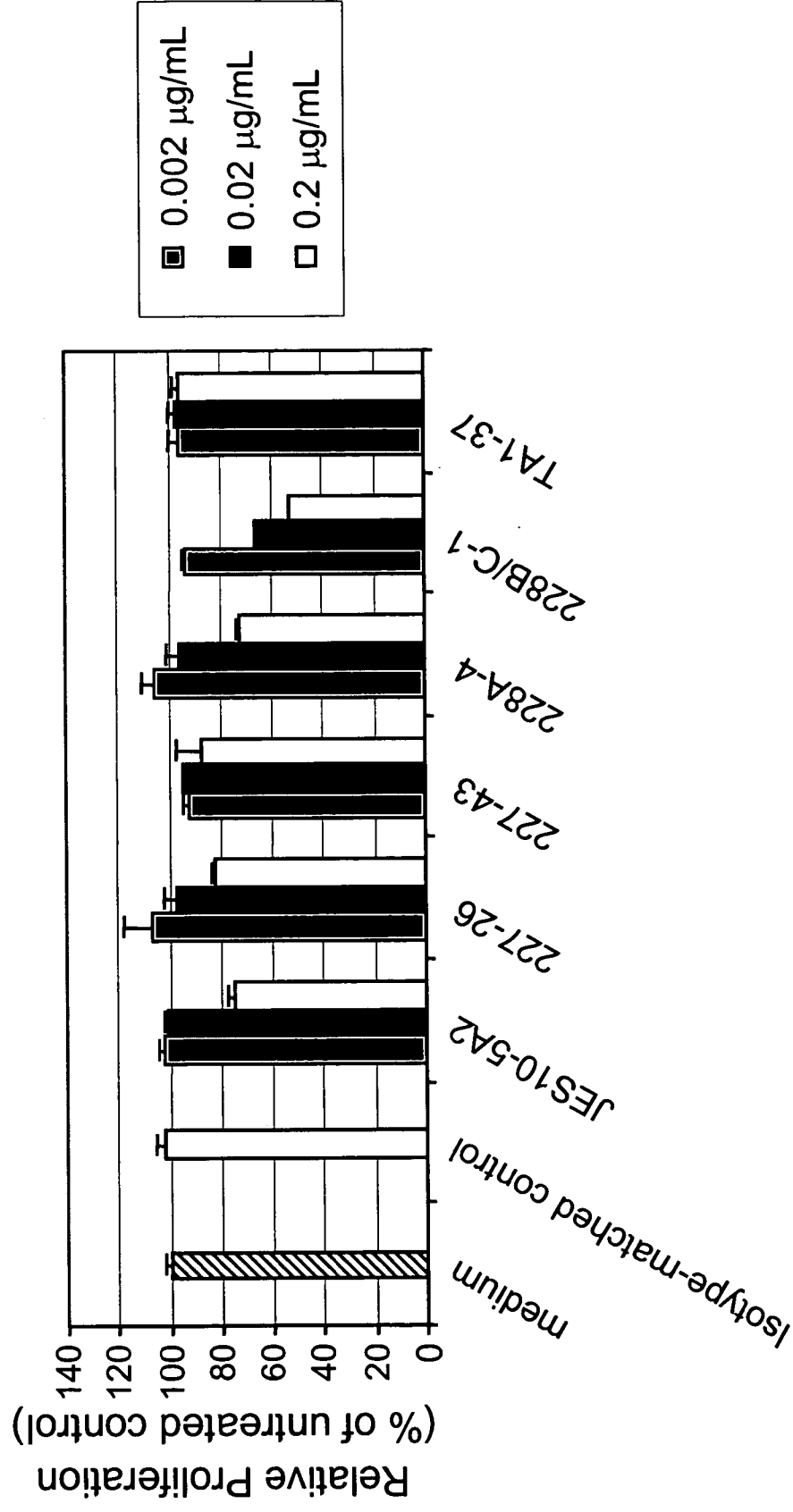


Fig. 5 Effect of anti-IL13 mAbs on IL13-induced suppression of CD14 expression in human monocytes

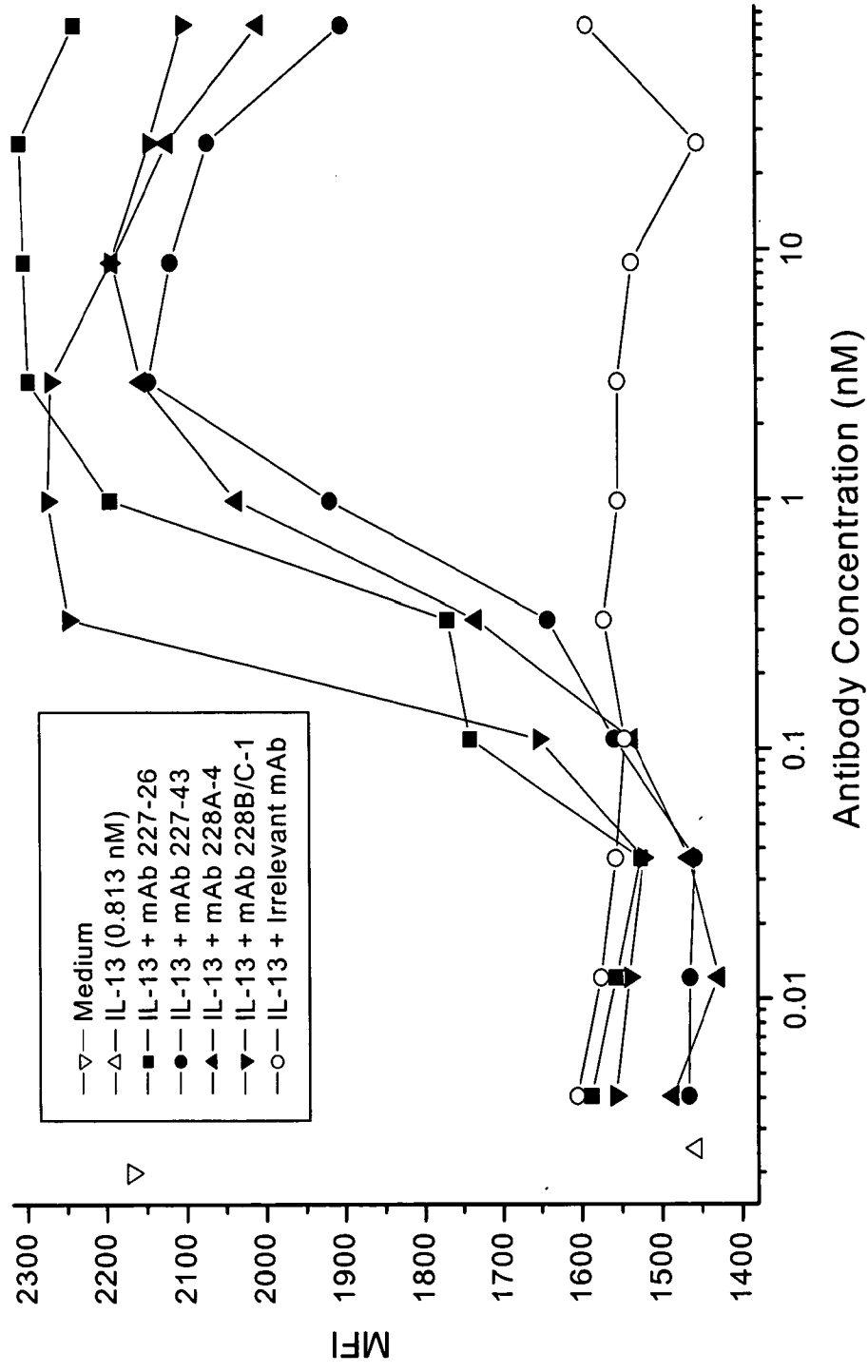


Fig. 6 Effect of anti-IL13 mAbs on IL13-induced upregulation of CD23 expression in human monocytes

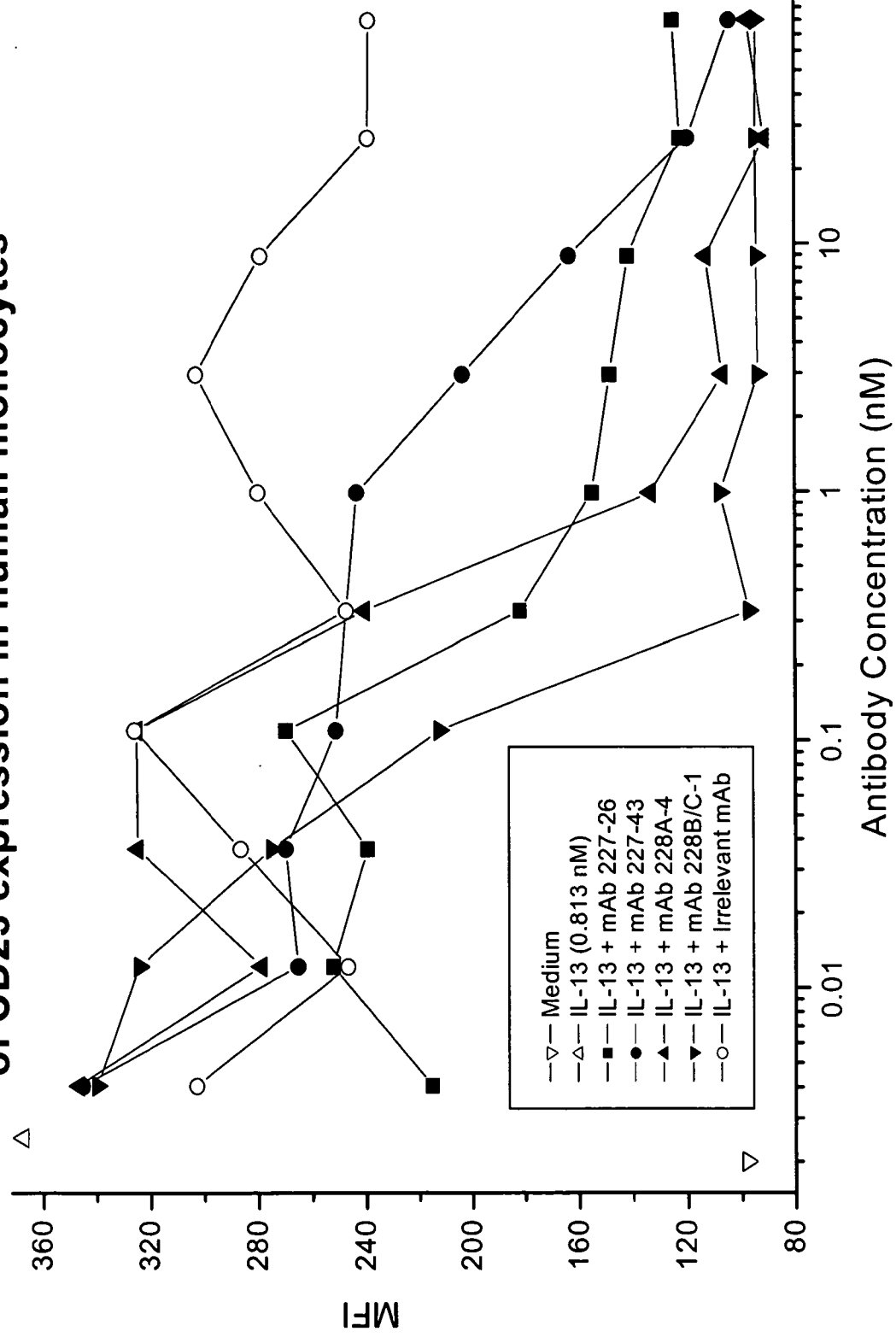


Fig. 7 Anti-IL13 mAbs Inhibit IL13-Induced Stat6 Phosphorylation in THP-1 Cells

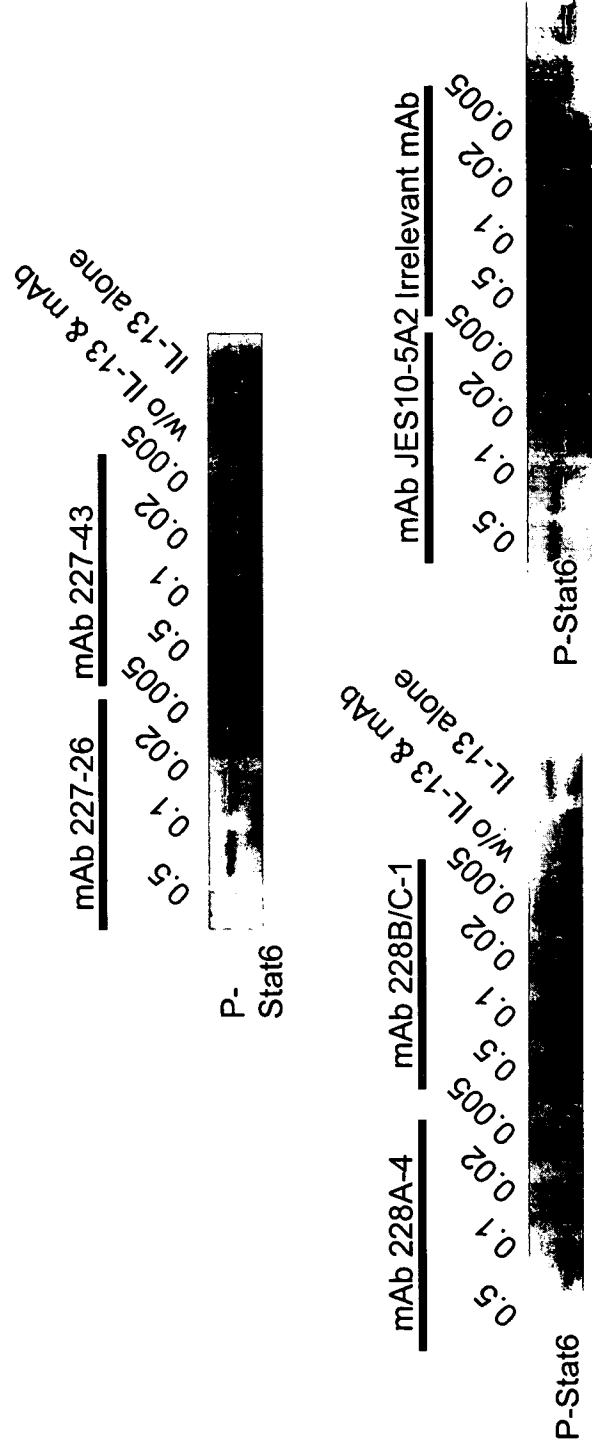


FIGURE 8

228B/C-1 Antibody Heavy Chain (VH)

1 10 20 30
Q V Q L Q E S G P G L V A P S Q S L S I T C T V S ***G F S L N A Y S V N W V***
40 50 52a 53 60 65 70
R Q P P G K G L E W L G ***M I W G D G K I V Y N S A L K S R L N I S K D S S***
80 82 90 95 100 b c d e f 101
K S Q V F L K M S S L Q S D D T A R Y Y C A G ***D G Y Y P Y A M D N W G H G T***
118
S V T V S S

Light Chain (VK)

10 20 30
N I V L T Q S P A S L A V S L G Q R A T I S C R A S K S V D S Y G N S F M H W
40 50 60 70
Y Q Q K P G Q P P K L L I Y L A S N L E S G V P A R F S G S G S R T D F T L
80 90 100 113
T I D P V E A D D A A S Y Y C Q Q N N E D P R T F G G G T K L E I K R A

Underlined: Kabat CDR. Bold/Italic: Chothia CDR1.

FIGURE 9
228A-4 Antibody
Heavy Chain (VH)

1	10	20	30
Q V Q L K E S G P G L V A P S Q S L S I T C T V S G P S L T D Y N I N W I R Q			
40	50 52a53	60	70
P P G K G L E W L G M I W G D G S T A Y N S A L K S R L S I S K D N S K S Q I F			
80 82ab c 83	90	95	100 b c d e f 101
L K M N S L Q T E D T A R Y Y C A R R D G Y F P Y A M A Y W G Q G T			
			118
			S V T V S S

Light Chain (VK)			
1	10	20	30
N I V L T Q S S P T S L A V S L G Q R A T I S C R A S E S V D S Y G N G F I H W			
40	50	60	70
Y Q Q K P G Q P P K L L I Y L A S N L E S G V P A R F S G S G S R T D F T L			
	80	90	100
			113
T I D P M E A D D A A T Y Y C Q Q N N E D P R T F G G G T K L E I K R A			

Underlined: Kabat CDR. Bold/Italic: Chothia CDR1.

227-26 Antibody
Heavy Chain (VH)

Light Chain (VK)

Underlined: Kabat CDR. Bold/Italic: Chothia CDR1.

SEQUENCE LISTING

<110> TANOX, INC. .
 FUNG, Sek Chung
 Moyle, Matthew
 LU, Meisheng
 YAN, Changning

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 20 25 30

Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala
 35 40 45

Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr
 50 55 60

Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln
 65 70 75 80

Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe
 85 90 95

Val Lys Asp Leu Leu Leu His Leu Lys Lys Leu Phe Arg Glu Gly Arg
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Phe Asn

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20 25 30

Ser Met Val Trp Ser Ile Asn Leu Thr Ala Gly Met Tyr Cys Ala Ala
35 40 45

Leu Glu Ser Leu Ile Asn Val Ser Gly Cys Ser Ala Ile Glu Lys Thr
50 55 60

Gln Arg Met Leu Ser Gly Phe Cys Pro His Lys Val Ser Ala Gly Gln
65 70 75 80

Phe Ser Ser Leu His Val Arg Asp Thr Lys Ile Glu Val Ala Gln Phe
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1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr
 20 25 30
 Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45
 Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asp
 65 70 75 80
 Pro Val Glu Ala Asp Asp Ala Ala Ser Tyr Tyr Cys Gln Gln Asn Asn
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 1 5 10 15
 Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Asn Ala Tyr
 20 25 30
 Ser Val Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45
 Gly Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys

50 55 60
 Ser Arg Leu Asn Ile Ser Lys Asp Ser Ser Lys Ser Gln Val Phe Leu
 65 70 75 80
 Lys Met Ser Ser Leu Gln Ser Asp Asp Thr Ala Arg Tyr Tyr Cys Ala
 85 90 95
 Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly His Gly Thr
 100 105 110
 Ser Val Thr Val Ser Ser
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 20 25 30
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 35 40 45
 Gly Met Ile Trp Gly Asp Gly Ser Thr Ala Tyr Asn Ser Ala Leu Lys
 50 55 60
 Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Ile Phe Leu
 65 70 75 80
 Lys Met Asn Ser Leu Gln Thr Glu Asp Thr Ala Arg Tyr Tyr Cys Ala
 85 90 95
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100

105

110

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115

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1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asp Tyr
20 25 30

Asn Ile Asn Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Met Ile Trp Gly Asp Gly Ser Thr Ala Tyr Asn Ser Ala Leu Lys
50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Ile Phe Leu
65 70 75 80

Lys Met Asn Ser Leu Gln Thr Glu Asp Thr Ala Arg Tyr Tyr Cys Ala
85 90 95

Arg Asp Gly Tyr Phe Pro Tyr Ala Met Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Ser Val Thr Val Ser Ser
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<222> (1)..(114)
<223> VARIABLE REGION OF LIGHT CHAIN OF MONOCLONAL ANTIBODY 227-26

<220>
<221> CHAIN
<222> (1)..(114)
<223> VARIABLE REGION OF LIGHT CHAIN OF MONOCLONAL ANTIBODY 227-26-1

<400> 7

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Gln Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg Ala

<210> 8
<211> 120
<212> PRT
<213> Murinae gen. sp.

<220>
<221> CHAIN
<222> (1)..(120)
<223> VARIABLE REGION OF HEAVY CHAIN OF MONOCLONAL ANTIBODY 227-26-1

<400> 8

Gln Val Gln Leu Gln Gln Ser Gly Asp Asp Leu Val Leu Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Trp Ile Asn Trp Ile Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly His Ile Ala Pro Gly Ser Gly Ser Thr Tyr Phe Asn Glu Met Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Ile Gln Leu Ser Ser Leu Ser Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Ser Asp Ile Phe Leu Ser Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 9
<211> 50
<212> DNA
<213> ARTIFICIAL

<220>
<223> Forward oligonucleotide primer for a mutant IL13 sequence

<400> 9
aagctttccc caggccctgt gcctccctct acagccctca ggaagctcat 50

<210> 10
<211> 30
<212> DNA
<213> ARTIFICIAL

<220>
<223> Reverse Oligo nucleotide primer of a mutant IL13 sequence

<400> 10
ctcgagggttg aaccgtccct cgcgaaaaag 30

<210> 11
<211> 22
<212> DNA
<213> ARTIFICIAL

<220>

<223> Forward degenerate oligonucleotide primer for monkey IL13

<400> 11
gyyctrggcy ycatggcgct yt 22

<210> 12
<211> 25
<212> DNA
<213> ARTIFICIAL

<220>

<223> Reverse degenerate oligonucleotide primer for monkey IL13

<400> 12
tttcagttga accgtccyty gcgaa 25

<210> 13
<211> 399
<212> DNA
<213> *Macaca fascicularis*

<400> 13
atggcgctct tgttgaccat ggtcattgct ctcaattgcc tcggcggtt tgcctcccca 60
agccctgtgc ctccctctac agccctcaag gagctcattg aggagctggt caacatcacc 120
cagaaccaga agggcccgtct ctgcaatggc agcatgggtg ggagcatcaa cctgacagct 180
ggcgtgtact gtgcagccct ggaatccctg atcaacgtgt caggctgcag tgccatcgag 240
aagaccaga ggatgtgaa cggattctgc ccgcacaagg tctcagctgg gcagttttcc 300
agcttgctg tccgagacac caaaatcgag gtggcccagt ttgtaaagga cctgctcgta 360
catttaaaga aacttttttcg caatggacgg ttcaactga 399

<210> 14
<211> 34
<212> DNA
<213> ARTIFICIAL

<220>

<223> Forward oligonucleotide primer for cynomologus monkey IL13

<400> 14
aagcttcacc atggcgctct tgttgaccat ggtc 34

<210> 15
<211> 40
<212> DNA
<213> ARTIFICIAL

<220>

<223> Reverse oligonucleotide primer for cynomologus monkey IL13

<400> 15

tcacaagatc tgggctcctc gaggttgaac cgtccattgc

40

<210> 16
<211> 23
<212> DNA
<213> ARTIFICIAL

<220>

<223> Forward oligonucleotide primer for Fc gamma1

<400> 16

ctcgaggagc ccagatcttg tga

23

<210> 17
<211> 35
<212> DNA
<213> ARTIFICIAL

<220>

<223> Reverse oligonucleotide primer for Fc gamma 1

<400> 17

gctctagagc ctcatttacc cggagacagg gagag

35